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**Investigating the Potential of Clinical Signs and Symptoms for Indicating
the Presence of Biofilm in Chronic Wounds: Development of a
Methodological Pathway.**

A thesis submitted to the College of Medicine, Nursing and Health Sciences, University of
Galway, Ireland in fulfilment of the requirements for the degree of Doctor of Philosophy.

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List of Abbreviations

µm = Micrometer

AGEs = Advanced glycation end products

AMP = Antimicrobial peptide

ASCVD = Atherosclerotic cardiovascular disease

BBWC = Biofilm-based wound care

bFGF = Basic fibroblast growth factor

CART = Classification and regression tree

CLI = Critical limb ischaemia

CLSM = Confocal laser scanning microscopy

COMET = Core Outcome Measures in Effectiveness Trials

CPR = Clinical Prediction Rule

CRDO = Clinical Research and Development Office

CSRF = Clinical Research Study Registration Form

CTA = Clinical Trial Agreement

CTR = Clinical Trials with Medicinal Products

DCF = Data collection form

DFD = Diabetic foot disease

DFI = Diabetic foot infection

DFU = Diabetic foot ulcer

DKA = Diabetic ketoacidosis

DM = Diabetes mellitus

DNA = Deoxyribonucleic acid

DPC = Data Protection Commission

DPIA = Data Protection Impact Assessment

DPO = Data Protection Office(r)

DSA = Data Sharing Agreement

DVT = Deep venous thrombosis

EBM = Evidence-based medicine

ECM = Extracellular matrix

EDTA = Ethylenediaminetetraacetic acid

EGF = Epidermal growth factor

EPS = Extracellular polymeric substance

EQ-5D = EuroQol-5D

ESCMID = European Society of Clinical Microbiology and Infectious Diseases

ESGB = ESCMID Study Group on Biofilms

ESRD = End-stage renal disease

EU = European Union

EuroSCORE II = European System for Cardiac Operative Risk Evaluation II

EWMA = European Wound Management Association

FGF = Fibroblast growth factor

GAD 65 = Glutamic acid decarboxylase 65

GADA = Glutamic acid decarboxylase autoantibodies

GCP = Good Clinical Practice

GCREC = Galway Clinical Research Ethics Committee

GDPR = General Data Protection Regulation

GMP = Good Manufacturing Practice

HBO = Hyperbaric oxygen

HIV = Human immunodeficiency virus

HLA = Human leucocyte antigen

HRB = Health Research Board

HRQoL = Health-related quality of life

HSE = Health Services Executive

IAA = Insulin autoantibody

ICA = Islet cell autoantibodies

ICF = Informed Consent Form

ICH = International Council on Harmonization

IDDM = Insulin dependent diabetes mellitus

IEC = Independent Ethics Committee

IGF = Insulin-like growth factor

IgG = Immunoglobulin G

IL = Interleukin

IMP = Investigational Medicinal Product

IRB = Institutional Review Board

IRIS = Institutional Research Information System

ISS = Information Solutions and Services

IVDR = *In Vitro* Diagnostic Regulation

IWGDF = International Working Group on the Diabetic Foot

IWII = International Wound Infection Institute

IWJ = International Wound Journal

JB I = Joanna Briggs Institute

JCA = Joint Controller Agreement

JREC = Joint Research Ethics Committee

JWM = Journal of Wound Management

KGF = Keratinocyte growth factor

LEA = Lower extremity amputation

MDR = Medical Devices Regulation

MMP = Matrix metalloproteinase

MODY = Maturity Onset Diabetes of the Young

mRNA = messenger Ribonucleic acid

MRSA = Methicillin Resistant *Staphylococcus aureus*

MSSA = Methicillin Susceptible *Staphylococcus aureus*

MTA = Materials Transfer Agreement

NGF = Nerve growth factor

NHS = National Health Service

NIDDM = Non-insulin dependent diabetes

NPV = Negative predictive value

OCT = Octenidine dihydro-chloride

PAD = Peripheral arterial disease

PCR = Polymerase chain reaction

PDGF = Platelet-derived growth factor

PEDF = Pigment epithelium-derived growth factor

pH = Potential of hydrogen

PHMB = Polyhexamethylene biguanide

PI = Principal Investigator

PIL = Participant information leaflet

PMN = Polymorphonuclear leucocyte

PNA = Peptide nucleic acid

PNA-FISH = Peptide nucleic acid fluorescence *in situ* hybridization

POC = Point-of-care

PPV = Positive predictive value

PRESS = Peer Reviewed Electronic Search Strategy

PRISMA = Preferred reporting items for systematic reviews and meta-analyses

PU = Pressure ulcer

R&I = Research and Innovation

REASSURED = Real-time connectivity, Ease of specimen collection, Affordable, Sensitive, Specific, User-friendly. Rapid and robust, Equipment-free or simple, Deliverable to end-users

REC = Research Ethics Committee

RNA = Ribonucleic acid

ROC = Receiver-operating characteristic

SD = Standard deviation

SEM = Scanning electron microscopy

SF-36 = 36-item Short Form Survey

SJH = St. James's Hospital

SUHG = Saolta University Hospital Group

T1DM = Type 1 diabetes mellitus

T2DM = Type 2 diabetes mellitus

TGF = Transforming growth factor

TIMP = Tissue inhibitor of metalloproteinases

TNF = Tissue necrosis factor

TUH = Tallaght University Hospital

UHG = University Hospital Galway

UK = United Kingdom

US = United States

USA = United States of America

USB = Universal serial bus

USD = United States dollars

USPHS = United States Public Health Service

UV = Ultraviolet

VBNC = Viable but nonculturable

VEGF = Vascular endothelial growth factor

VLU = Venous leg ulcer

WHO = World Health Organisation

WHOQOL-bref = World Health Organization Quality of Life-BREF

WMA = World Medical Association

Abstract

Introduction

Chronic wounds are injuries to the skin that fail to proceed to closure through the healing processes of haemostasis, inflammation, proliferation and maturation/modelling. Failure of the healing pathway usually occurs because of comorbidities such as diabetes, venous insufficiency, or circumstances of adverse pressure on areas of the skin.

Biofilms are an aggregate or community phenotype exhibited by bacterial cells that are ubiquitous in the environment. Aggregates are three dimensional, affixed to/associated with biotic/abiotic surfaces and surrounded by a self-produced extra cellular polymeric substance. Furthermore, the aggregates exhibit internal physiological gradients and are extremely resistant to adverse environmental circumstances including host immune responses and antimicrobial therapy.

It is generally accepted that biofilms contribute to delayed healing of chronic wounds and there are interventions available to mitigate their adverse effects. However, evidence supporting these interventions is limited. Furthermore, identification of biofilm is challenging; techniques are not routine, are of limited ability or are still in early stages of development.

These limits in terms of evidence to support interventions for treating and techniques for identifying biofilm give rise to concerns: if biofilm cannot be readily confirmed in a wound, how can it be known that it is there in the first place, if interventions have been effective and have removed/destroyed it, and if its removal/destruction truly benefits the wound.

A means to address these concerns could be to create a predictive tool that is readily capable of indicating presence of biofilm in chronic wounds in a clinical setting.

A Clinical Prediction Rule is a process by which the probability of a clinical condition or outcome is estimated based on the presence or absence of valid predictive factors or indicators which could comprise clinical signs, patient characteristics or investigative results.

Clinical signs and symptoms are commonly used to confirm biofilm presence in chronic wounds and algorithms that incorporate them have been developed. However, there is little evidence to support their use in this fashion.

Aim

The aim of this PhD thesis is to begin directing research in wound care toward efforts to develop a convenient tool that would incorporate statistically validated clinical signs and symptoms to facilitate prediction of biofilm in lower-limb ulcers secondary to type two diabetes.

Methods

The methodology for this PhD thesis draws on the derivation stage of the clinical prediction rule approach and consists of five phases. A scoping review of the literature was conducted to collate a list of all clinical signs and symptoms reported therein to be indicative of biofilm in chronic wounds. A two-round, global electronic Delphi consensus study brought a panel of experienced wound care clinicians together to refine the list resulting from the scoping review through expert clinical opinion. The refined list from the electronic Delphi study was incorporated into protocol for a clinical study designed to estimate their true statistical predictive abilities. A guidance document was created to facilitate navigation through regulatory, ethical and data protection systems and gain approved access to an investigative site. A laboratory procedure to identify biofilm morphological features that uses a series of fluorescent dyes in conjunction with confocal laser scanning microscopy was developed and subjected to preliminary *in vitro* testing.

Results

The scoping review included 111 reports of 109 articles that met eligibility criteria. These reports yielded 830 accounts of a clinical sign advocated for use as an indicator of biofilm in chronic wounds. These accounts were categorised into a final list of 26 clinical sign and symptom items. The electronic Delphi study brought together 103 wound care clinicians from 22 countries, the majority of whom came from the nursing profession and had more than 10 years' experience in a wound care environment. This panel voted to retain eight of the 26 items from the scoping review at the end of round one and three more at the end of round two. All other items were left with a status of 'no consensus' at the end of two rounds.

Visual indicators of biofilm in chronic wounds (a shiny, slimy layer on the wound bed that is easily removed but returns quickly in the absence of frequent intervention) received the highest percentage of votes to retain. The clinical study protocol received ethical approval from Galway

Clinical Research Ethics Committee, and the guidance document will serve novice or inexperienced researchers at the University of Galway, facilitating progress of an observational clinical study through the systems that grant approved access to an investigative site. This document was reviewed by members of the Trials Accelerator group of the Institute for Clinical Trials at the University of Galway and they plan to incorporate it into their future practices. The laboratory procedure demonstrated itself capable of highlighting cell aggregates and extracellular polymeric substance components of biofilm under *in vitro* conditions that are commonly used in research to confirm its presence in wound tissue biopsies.

Conclusion

Each methodological phase of this PhD thesis resulted in a valuable research output that can contribute much to research in wound care. However, when taken together, their contribution becomes much more significant. For the first time the aggregated outputs of this PhD thesis have opened an avenue for research efforts to be directed into a clinical prediction rule project to determine the possibility of developing a readily available, statistically validated predictive tool that uses clinical signs and symptoms to indicate the presence of biofilm in chronic wounds.

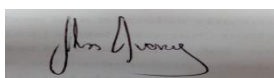
Declaration

I, John Damien Ivory declare that this work is my own and that it is being submitted to the University of Galway to fulfil requirements for the degree of Doctor of Philosophy. I have not submitted it to this institution nor to any other academic institution for another degree.

I am also the principal author of the four included published manuscripts. Contributions by others are included under ‘Contributions to Research’.

All external references and sources are acknowledged and identified within the content.

Signed:

A handwritten signature in black ink, appearing to read 'John Ivory', is written over a light grey rectangular background.

Date: September 25th 2025

Publications and presentations arising from this thesis

Publications

Ivory, J.D., Sezgin, D., Coutts, P.M., Roshan, D., Hobbs, C.M., Soriano, J.V., O’Gara, J.P., Gallagher, D. & Gethin, G. (2025). Clinical signs and symptoms of biofilm in chronic wounds. What do practitioners think? Consensus through an electronic Delphi Survey. *International Wound Journal*. 22(11), e70771.

Ivory, J.D., Perrier, L., Vellinga, A., Sezgin, D., Hobbs, C.M., Ffrench, C., Coutts, P.M., O’Gara, J.P., & Gethin, G. (2025). A scoping review to identify clinical signs, symptoms and biomarkers reported in the literature to be indicative of biofilm in chronic wounds. *International Wound Journal*. 22(5), e70181.

Ivory, J.D., Sezgin, D., Coutts, P.M., Roshan, D., Hobbs, C.M., Soriano, J.V., O’Gara, J.P., Gallagher, D. & Gethin, G. (2023). Signs, symptoms and/or biomarkers reported to indicate biofilm in chronic wounds: an eDelphi consensus protocol. *Journal of Wound Management*. 24(1), 22-27.

Ivory, J.D., Vellinga A., O’Gara, J.P. & Gethin, G. (2021). A scoping review protocol to identify clinical signs, symptoms and biomarkers indicative of biofilm presence in chronic wounds [version 2; peer review: 2 approved, 1 approved with reservations]. *HRB Open Res*. 4:71.

Conference presentations (oral)

34th Conference of the European Wound Management Association (EWMA) in collaboration with the Society of Tissue Viability. London, UK May 1st – 3rd 2024. ‘Clinical signs, symptoms and biomarkers indicating presence of biofilm in chronic wounds. A scoping review.’

5th Transatlantic Wound Science and Podiatric Medicine Conference/All Ireland Podiatry Conference, Galway, Ireland April 28th – 30th 2022. ‘Understanding biofilms in the context of chronic wounds.’

Diabetic Foot Disease: From Prevention to treatment to Improved patient Outcomes (DFD PRIMO) conference. Virtual June 10th, 2021. ‘A scoping review protocol to identify signs and symptoms indicative of biofilm presence in chronic wounds.’

Conference presentations (poster)

The 35th conference of the European Wound Management Association (EWMA) in cooperation with GNEAUPP – The Spanish Advisory Group for the Study of Pressure Ulcers and Chronic Wounds. Barcelona, Spain March 26th -28th 2025. ‘Clinical Signs and symptoms of biofilm in chronic wounds. What do practitioners think? Consensus through an electronic Delphi survey.’

The 33rd conference of the European Wound Management Association (EWMA). Milan, Italy May 3rd – 5th 2023. ‘Clinical signs, symptoms and biomarkers indicating presence of biofilms in chronic wounds: a scoping review.’

Contributions to Research

This thesis consists of five manuscripts, an ethically approved protocol for a clinical study and a guidance document all of which are presented in the methods and results chapter. Four of these manuscripts have been published in peer reviewed wound care journals and a fifth will be submitted to a peer reviewed wound care journal. The clinical study protocol has received ethical approval from Galway Clinical Research Ethics Committee (GCREC). The guidance document has been reviewed by the Trials Accelerator group at the University of Galway's Institute for Clinical Trials, and they plan to incorporate it into their future practices.

The first manuscript consists of a methodological protocol for a scoping review of the literature that aimed to collate a list of clinical signs and symptoms reported therein to be indicative of biofilm in chronic wounds. I conceptualised and designed the protocol with guidance from Professor Gethin, Professor O'Gara and Dr. Akke Vellinga. I wrote the draft manuscript for publication, circulated it to coauthors and subsequently incorporated their suggested edits. I was responsible for seeing it through the HRB Open Research open access publishing platform's submission process wherein I made the initial submission, addressed peer reviewer concerns which were reviewed by Professor Gethin, and I submitted the revised manuscript.

The second manuscript is a report of the execution of this scoping review and its findings. I was responsible for coordinating the efforts of the eight colleagues who assisted with screening of titles and abstracts and full text articles. I invited these colleagues to come on board, arranged pilot screening exercises and follow up meetings thereof and assigned tranches of titles and abstracts and full text articles to these colleagues on the basis of their availability. I screened all titles and abstracts and full text articles as a duplicate screening partner with each colleague. I located full text articles online, through efforts to contact authors and with assistance from The University of Galway's James Hardiman Library Inter Library Loans service. I managed the storage and tracking of all titles and abstracts and full text articles as they passed through the review to permit an accurate PRISMA flow diagram and tables of included and excluded studies to be created. I extracted data from all reports included in the study and processed that data for presentation in a published manuscript. I wrote the draft manuscript for publication, circulated it to coauthors and subsequently incorporated their suggested edits. I was responsible for seeing it through the International Wound Journal's submission process wherein I made the

initial submission, addressed peer reviewer concerns which were reviewed by Professor Gethin and submitted the revised manuscript.

The third manuscript consists of a methodological protocol for an international electronic Delphi consensus study to refine the findings of the scoping review by a panel of experienced wound care clinicians. There are dilemmas and uncertainties in regard to aspects of Delphi methodology and Professor Gethin suggested that we convene a steering committee to examine them and help devise solutions that would optimise the rigour of this study's methodology. We selected an international membership of nine experienced clinicians and researchers including ourselves, and I convened and chaired a meeting of this committee. I created the agenda for this meeting which was based on a series of these methodological dilemmas and ensured that the committee proceeded through them in a timely fashion. I subsequently reviewed the insights and suggestions presented during the meeting and used them to write the methodology. I circulated the methodology to the committee for review and incorporated their suggested edits. I received assistance from Dr. Davood Roshan at the University of Galway's School of Mathematical and Statistical Sciences with the statistical component of the methodology. I wrote the draft manuscript of the protocol for publication, circulated it to coauthors and subsequently incorporated their suggested edits. I was responsible for seeing it through the European Wound Management Association's (EWMA) Journal of Wound Management submission process wherein I made the initial submission, addressed peer reviewer concerns which were reviewed by Professor Gethin and submitted the revised manuscript.

The fourth manuscript is an account of the execution of the electronic Delphi survey and its findings. I contacted 18 international wound care organisations with requests for help in circulating the questionnaire for this survey to their membership. I designed the survey, circulated it to steering committee members and incorporated their suggested edits and changes. I developed the online version using the QuestionPro survey platform. I wrote the invitation and reminder emails and participant information leaflets for potential participants (organisation members) which were reviewed by Professor Gethin and other committee members. I prepared the submission package for the University of Galway Research Ethics Committee and completed the ethics application form. I liaised with organisation gatekeepers to ensure that invitation emails were circulated in a timely fashion. I managed the online questionnaire and when it was completed I collated all data and processed it. I received

assistance from Dr. Davood Roshan at the University of Galway's School of Mathematical and Statistical Sciences with the statistical component of the data processing. I wrote the draft manuscript for publication, circulated it to coauthors and subsequently incorporated their suggested edits. I was responsible for seeing it through the International Wound Journal's submission process wherein I made the initial submission, addressed peer reviewer concerns which were reviewed by Professor Gethin and submitted the revised manuscript.

The fifth manuscript is an account of the development and preliminary *in vitro* testing of a laboratory procedure that uses fluorescent dyes to highlight morphological features of bacterial biofilms. I grew Methicillin resistant *Staphylococcus aureus* and Methicillin susceptible *Staphylococcus aureus* biofilms from isolates taken from diabetic foot ulcer swabs under the close supervision of Dr. Aaron Nolan in the laboratories of Microbiology, School of Biological and Chemical Sciences at the University of Galway and stained these biofilms with a series of fluorescent dyes specific for DNA and biofilm extracellular matrix components, also under the supervision of Dr. Nolan. Confocal Laser Scanning Microscopy (CLSM) for which I do not have training was carried out by Dr. Nolan and Dr. Merve Zeden at the University of Galway's Anatomy, Imaging and Microscopy facility and I uploaded the resulting images to ImageJ (a java-based image processing program) and processed them for presentation in the manuscript. I wrote the draft manuscript for publication, circulated it to coauthors and subsequently incorporated their suggested edits. I will undertake to submit this manuscript to a peer reviewed wound care journal.

The clinical study protocol was designed to statistically determine the true predictive abilities of a series of clinical signs and symptoms thought to be indicative of biofilm in chronic wounds. In this study the wounds of consenting patients with lower limb ulceration secondary to type two diabetes will be assessed for presence of each sign and symptom in the series. A gold standard biopsy will be taken from each assessed wound which will be examined in the laboratory for presence of biofilm. Results of biopsies and wound assessments will be matched, and diagnostic statistics will be calculated for each sign and symptom, that is, sensitivity, specificity, discriminatory power and positive predictive value. I wrote the methodology, procedures and statistical processes for this study based on similar work in other medical fields and on my own previous experience with clinical trials. Co-investigators on this study reviewed and approved the protocol. I also wrote the participant information leaflet and informed consent

form, the care provider follow-up letter and data collection form which were reviewed by Professor Gethin. I prepared the submission package for Galway Clinical Research Ethics Committee (GCREC) and completed the ethics application form. I also addressed board queries relating to the application. In addition, I prepared an amendment to the protocol following consultation with personnel from the Dermatology Department at University Hospital Galway (UHG) and submitted to GCREC.

The guidance document arose as a result of my journey through the regulatory, ethical and data protection systems that govern access to investigative sites for clinical trials and grant approval for them to proceed.

Initially this clinical study was intended to proceed at the Diabetes Centre, University Hospital Galway. I presented the study protocol to personnel at this centre and gained their provisional approval to host the study there. I arranged for ethical approval as described above, I liaised with the UHG supplies office to arrange for study materials and worked on resolving invoicing issues between UHG and the University of Galway. I organised secure storage facilities for study documentation at the Diabetes Centre and biopsy samples at the University of Galway's Microbiology, School of Biological and Chemical Sciences. I also consulted with personnel at the Dermatology Department of UHG to resolve outstanding issues pertaining to biopsies in this study's population, optimising the procedure to make it as safe and efficient as possible and in keeping with UHG standard practice.

Logistical issues at the Diabetes Centre at UHG meant that they were forced to suspend all research activities there and so an alternative investigative site had to be located. A colleague at the Diabetes Day Centre at St. James's Hospital (SJH) was suggested and I contacted them. The Diabetes Day Centre offered to host this study at their facility, and I subsequently initiated contact with the appropriate regulatory, ethical and data protection systems to gain approved access for this study at that site. I prepared a submission package to the St. James's Hospital/Tallaght University Hospital Joint Research Ethics Committee (SJH/TUH JREC) and completed their online application form. I also completed an online application form for the SJH Research and Innovation Office (R&I). At the University of Galway, I engaged with the Data Protection Office (DPO), completing and submitting a Data Protection Impact Assessment form. I addressed queries to this form, meeting with DPO personnel in the process. I met and engaged with personnel from the Clinical Research and Development Office to arrange to have this study registered with them. I engaged with the Insurance Services Office

to ensure that the study had full indemnity coverage, and I engaged with the Institute for Clinical Trials, Trials Accelerator group who assisted with navigating these systems. I also convened and chaired a meeting with the DPO and Trials Accelerator group in relation to defining roles and necessary documentation in terms of data protection for this study.

I documented the experiences I had while working within these systems and wrote a novel guidance document that includes a schematic map which will serve to guide novice researchers in the future through these systems in as timely and efficient a manner as possible.

Acknowledgements

A lot of people have provided a lot of support and have contributed much of their time, experience, insights and knowledge to this PhD thesis without which it could not have gotten completed. Thankfully, I have been keeping a list and updating it as I go, so hopefully I will be able to include everyone.

First of all, I would like to extend deepest thanks and gratitude to my supervisors, Professor Georgina Gethin and Professor James P. O’Gara who provided constant professional and academic support and guidance to me throughout the duration of this project. I would especially like to thank them for the kindness and understanding that they demonstrated during the tougher and more challenging times.

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Gratitude and appreciation is very much due to the faculty of the Collaborative Doctoral Awards, Diabetic Foot Disease: From Prevention to Improved patient Outcomes (DFD PRIMO) program, Professor Georgina Gethin, Professor Timothy O’Brien, Professor Caroline McIntosh, Professor Sean Dineen, Professor Claire Buckley at University College Cork, Professor James P. O’Gara, Professor Gerard O’Connor, Professor John Newell, Professor Brian McGuire, Dr. Yvonne Finn, Dr. Jane Sixsmith and Professor Paddy Gillespie. You worked extremely hard to make this program a reality and you actively participated in it to make it a valuable and rewarding experience. Thank you for taking me under your collective wing.

Staying with the DFD PRIMO program, I must mention Dr. Chloe Hobbs, Ms. Michelle Hanlon, Mr. Enda Naughton, Dr. Lauren Connell, Dr. Jennifer Pallin at University College Cork, Ms. Sinead Flynn and Ms. Isha Sikri, my fellow students. I consider myself fortunate to find myself working and learning with such a great group of people as you. Thank you for your support, collaboration and companionship. I hope we will get to work together in some shape or form again.

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Now, digging down into the day-to-day research work there are a lot of people who deserve to be remembered.

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Now it’s time to take a brief visit back to my roots in wound care and the evidence synthesis days.

Tonya Eggleston RN. You are responsible for all of this! I often wonder where I would be now had we not started talking on the train to Dublin way back in 1996 (chapter 1, section 1.2). Thanks for introducing me to the team at the Toronto Women’s College and Mississauga wound care clinics and for being so instrumental in getting this big old ball rolling.

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Finally, on a more personal note I would like to look to my family and friends. My late parents John and Eileen (Eileen, I left a special typo in chapter 4 for you!), my brothers Mark, Paul and Brian, their wives Deirdre, Niamh and Kathleen, my favourite nephew Ben, my favourite eldest niece Aoibheann and my favourite youngest niece Ellen, Tracy and her family, and Damien, and friends Rory and Trish, Ethel, Oliver, Tom and Brady, Jonathan and Rocio, Brian, Tom, Maura, O'Higgins, Shyne-o-myte, Petey, Matt, Geoffo, Jho (chapter 1, section 1.2), Fiona, Eamonn, Sandra, Eddie and Aidan, Allan, Courtney, Mark, Patrick, Carlos, Pedro, Jesus (not that one), Phil, Gillian, Siobhan, Josephine, Sabrina, Fr. Patrick Kiernan PP, St. Rynagh's parish, Fr. Ben and the Chaplaincy here at University of Galway and Dr. Bernadette Earley, Teagasc.

Thank you for being there, for the companionship, the support, the fun, the conversations, the gatherings, the Christmases and for showing a genuine interest in this work as it progressed.

Dedication

I would like to dedicate this thesis to all those individuals with chronic wounds and to the clinical, research and administrative people who work so hard to help them work towards wellness.

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^{*} The tables and figures that contain data created through the execution of the methodological phases of this thesis have been included in manuscripts that have been published, are awaiting publication or will be submitted for publication in the near future.

The methods and results chapter of this thesis consists of a series of these manuscripts, presented as they were prepared, each of which has its own results section in which tables and figures are numbered and addressed accordingly.

To present them as such in this list would mean having multiple figures and tables with the same number which could prove confusing. Therefore, only those tables that appear in thesis sections other than the methods and results chapter are listed.

1 Chapter One: Thesis introduction

1.1 Introduction

This brief chapter will introduce the following doctoral thesis and myself, its author to the reader. The journey that brought me to this project will be described along with how the experiences gained along the way shaped me and provided a set of skills that put me in an ideal position to undertake the work required to complete it. It will also present the thesis, providing a brief background and rationale, and it will conclude with its aims and a description of its structure.

1.2 Background of the author

Synchronicity is a strange phenomenon, and I can't help but wonder about how big a role it plays in our lives if we let it. Does synchronicity really set us up to have encounters with unexpected people, to travel down unexpected avenues and to experience unexpected adventures and exploits, all of which link together and form pathways to unexpected outcomes that can only be perceived in retrospect?

I wonder if this PhD thesis might have been the product of synchronicity, and I will leave it up to you the reader to agree or disagree with me after reading these next paragraphs.

I hold a bachelor's degree in biochemistry and a master's degree in neuropharmacology, which I originally perceived as pointers towards a more pre-clinical, bench-oriented professional life. However, I believe that apparently random encounters with two individuals may have changed all that, initiating a series of encounters and experiences (unplanned by me) that would culminate in this PhD thesis. The first encounter was with an American tourist on the train from Tullamore to Dublin back in September of 1996. She was an intensive care nurse based in Baltimore, Maryland, and a conversation on the train and time spent exploring Dublin together started a friendship that is still strong today. Another significant friendship with a Canadian from Mississauga, Ontario that began out of an apparently random encounter some two years later would also prove to be instrumental.

Fast forward some years and my Canadian friend suggested that I come to Toronto and spend some time there, ostensibly to play music with him and I subsequently found myself living there and indeed playing a little music. At the same time, I began looking around to see if I could find some work in medical research. I remember sending countless resumes to countless human resource departments, but no doors seemed to be opening in this area. However, my American tourist friend, who had since left nursing to become a clinical trials monitor, was assigned to provide oversight on a randomized controlled trial of a wound dressing at two chronic wound clinics in Toronto and Mississauga. She introduced me to one of the principal investigators, Dr. R. Gary Sibbald and to cut a long story short, I spent three years with his group, gaining a solid grounding in both wound care and clinical trials methodology.

At the time, Dr. Sibbald was working on the WoundPedia website and had enlisted the help of a librarian from the University of Toronto who also became a close friend. Three years after returning to Ireland, she contacted me out of the blue and asked me if I might be interested in working with the Li Ka Shing Knowledge Institute at St. Michael's hospital, Toronto on their systematic review program. I accepted and spent the next four years working online with Dr. Andrea Tricco and her team at the institute where I gained valuable experience in the systematic review process, helping to develop systematic reviews of interventions including cognitive enhancers for Alzheimer's disease, cardiopulmonary resuscitation, insulin analogs and antiretroviral therapy for Human immunodeficiency virus (HIV).

In early 2017 I saw an advertisement for a research assistant position in wound care with Professor Georgina Gethin in the school of Nursing and Midwifery at the now University of Galway. I applied and interviewed for the job but was not accepted. However, the successful applicant left early in the contract and Professor Gethin contacted me and asked me if I would be interested in filling the vacancy. The three years I spent in this position further consolidated my knowledge of chronic wounds, I engaged in more evidence synthesis work, and I began to expand my professional network.

The opportunity to take on this PhD project came about through Prof. Gethin during 2019 - 2020 and in the following paragraphs I will provide a brief background to it in terms of the population and concept of its research question, along with a rationale for the work and

description of its methodological processes into which I will link my own professional experiences.

1.3 Thesis population

The population of interest for this project is persons with foot ulceration secondary to type two diabetes. Global estimates for diabetic foot ulcer (DFU) prevalence vary (McDermott et al., 2023). The International Diabetes Federation estimates that 40 – 60 million people currently have a DFU but a meta-analysis from 2017 estimated that 33 million people suffer from the condition or a 6.3% global prevalence (Zaidi et al., 2024, Zhang et al., 2017). The annual incidence has been reported to be between 9.1 to 26.1 million people, the lifetime rate of ulcer occurrence is thought to range between 12 and 25% and the overall recurrence rate has been estimated to be 22.1% per person-year (Zaidi et al., 2024). Foot ulceration in diabetes develops out of neuropathic, ischaemic and mechanical pathophysiological pathways (Packer et al., 2025, Armstrong et al., 2023). Sensory, motor and autonomic neuropathy leads to loss of sensation for pain, pressure and temperature as well as impaired proprioception, wasting of small muscles, deformities and biomechanical anomalies, and sympathetic autonomic dysfunction leads to a decrease in sweat production and excessive skin dryness (Armstrong et al., 2023). Mechanical stressors can take the form of continuous low pressure damage, caused for example by ill-fitting shoes, repetitive stress caused by abnormal pressure during weight bearing or direct mechanical trauma often caused by a foreign body in the shoe, and peripheral artery disease results in reduced perfusion in the lower extremities which eventually leads to chronic limb-threatening ischaemia (Armstrong et al., 2023). Callus tissue can build up because of structural changes in the foot and unnoticed minor trauma and inflammation caused by repeated impacts from weight bearing can cause damage beneath the callus which will ultimately present as a full thickness ulcer (Armstrong et al., 2023). Impaired perfusion of the lower extremities leads to reduced supply of oxygen and nutrients to the wound site, further complicating the healing process (Aljohary et al., 2024).

1.4 Thesis concept

The concept of interest for this project is infection in diabetic foot ulceration, in particular infections thought to be caused by bacteria in the biofilm phenotype (which will be described below), and confirmation of their presence in this ulcer type.

Infection in diabetic foot ulcers

Infection is a grave complication of DFU, affecting 50 - 60% of these wounds and bringing increased risk of morbidity and poor clinical outcomes (Pickwell et al., 2015, Ivory et al., 2018, Hobizal and Wukich, 2012, McDermott et al., 2023). Infected foot ulcers are the most common diabetes-related cause of hospitalization, accounting for 20% of all admissions and 10 - 45% of patients hospitalised for a diabetic foot infection (DFI) will require readmission within 12 months (McDermott et al., 2023, Hobizal and Wukich, 2012). Fifteen to 20% of moderate to severe infections in diabetic foot ulcers result in a lower extremity amputation and patients with an infected DFU run a 150 fold increased risk of a lower extremity amputation versus patients with non-infected DFU (Armstrong et al., 2023, Ivory et al., 2018, Pickwell et al., 2015). Furthermore, 16.67% of patients with a DFI will die within 12 months (Hobizal and Wukich, 2012).

Biofilms

Biofilms can be found in diverse media such as dental plaque, public health environments, water systems, implantable devices and living tissue and they comprise three-dimensional aggregates of microbial cells that surround themselves in a self-produced extra cellular matrix (ECM) composed of polysaccharides, proteins, lipids and DNA (Zhao et al., 2023, Percival et al., 2015a, Hurlow et al., 2015, Lichtenberg et al., 2023, Bjarnsholt et al., 2013, Metcalf et al., 2014, Yin et al., 2019, Donlan, 2002, Khatoon et al., 2018). This phenotypic form allows bacterial cells to manage their environment and to protect themselves from adverse environmental challenges such as temperature and pH variations, nutritional shortfalls, host defence systems and antimicrobial treatment (Percival et al., 2015a, Yin et al., 2019). There is consensus that biofilm significantly delays wound healing (Goswami et al., 2023, Schultz et al., 2017). Hurlow and Bowler indicate that bacterial biofilms are a root cause of chronic wound infections and it has been hypothesised that wound biofilms commandeer the host immune response, locking the wound in a hyper-inflammatory state which provides a consistent source of nutrients derived from excessively produced exudate (Hurlow and Bowler, 2022, Wolcott et al., 2008).

Treating biofilms in chronic wounds

Biofilm infections in chronic wounds are typically treated with strategies that include sharp debridement, systemic or topical antimicrobial therapy, use of wound cleansers and antimicrobial dressings (Metcalf et al., 2014, Cavallo et al., 2024, Razdan et al., 2022). Biofilm-based wound care (BBWC) is a regimen of repeated sessions comprising wound hygiene,

debridement, therapeutic cleansing and topical antimicrobials/antimicrobial wound dressings with the aim of removing the more tolerant microorganisms from the wound bed and preventing or delaying biofilm reformation (Swanson et al., 2022). Cleansing agents include surfactants, super-oxidised solutions and povidone iodine, and antiseptic agents reported to have antibiofilm properties include polyhexamethylene biguanide (PHMB), medical honey and silver (Swanson et al., 2022). Other agents considered to have antibiofilm properties include quorum sensing inhibitors, ECM degrading enzymes, metal chelators and antimicrobial peptides (Razdan et al., 2022). However, these strategies are subject to query. Use of systemic antibiotics in diabetic foot ulcers is not always the preferred option and systemic treatment comes with risks of adverse effects, adverse drug reactions and inefficient delivery to the site of infection (Razdan et al., 2022, Attinger and Wolcott, 2012). Topical antibiofilm agents are non-selective, they can be toxic to host tissue and much of the evidence in their favour comes from *in vitro* and animal studies (Razdan et al., 2022, Swanson et al., 2022, Weigelt et al., 2021, Wolcott and Rhoads, 2008). Furthermore, there is no consensus on wound cleansing techniques and there are inconsistencies in aseptic techniques and use of antiseptic solutions (Swanson et al., 2022).

Detecting biofilms

Biofilms are difficult to detect and there are no indisputable diagnostic tests currently available (Høiby et al., 2015, Wu et al., 2019a). Options include semi-quantitative cultures obtained with swab techniques, metagenomics using 16s ribosomal RNA or DNA to identify pathogens in biofilm form, advanced microscopy including scanning electron microscopy (SEM) and confocal laser scanning microscopy (CLSM), peptide, nucleic acid fluorescence in situ hybridisation (PNA-FISH) – a labelling technique using fluorescently tagged peptide nucleic acid probes that can specifically bind bacterial ribosomal RNA, and other novel techniques such as quantification of biofilm associated biomarkers or wound blotting with nitrocellulose membranes which are then stained for ECM components (Darvishi et al., 2021, Oates et al., 2014, Fazli et al., 2009, Høiby et al., 2015, Kirketerp-Møller et al., 2008, Wu et al., 2019a). According to the European Society for Clinical Microbiology and Infectious Diseases (ESCMID), the advanced microscopy methods are most appropriate for detecting biofilm in biopsies of wounds and they also consider PNA-FISH and fluorescence microscopy to be acceptable (Høiby et al., 2015). However, standard culturing can only identify a miniscule percentage of bacteria present because of viable but non-culturable cells (VBNC) in the biofilm and an inability to capture biofilms located below the wound surface, microscopy techniques

are highly specialised and not available for routine use in clinical microbiological laboratories or in clinical settings, and other techniques described are novel and still in the early stages of development (Wu et al., 2019b, Høiby et al., 2015, Johani et al., 2017, Percival et al., 2015b, Wei et al., 2019).

A consensus guideline from 2017 recommended that specific signs and symptoms should be used to confirm biofilm presence in chronic wounds in the absence of diagnostic tests in the clinical setting (Schultz et al., 2017). This guideline and principals for best practice for wound infection by the International Wound infection Institute (IWII) both provide lists of signs and symptoms, for example delayed wound healing, recurrent infection, excess exudate, chronic inflammation and antimicrobial failure that are potentially indicative of biofilm in chronic wounds, and there are numerous instances in the literature where lists of clinical signs and symptoms are reported as being indicative of biofilm in chronic wounds (Schultz et al., 2017, Swanson et al., 2022, Ivory et al., 2025a). However, the abilities of some signs to indicate biofilm in wounds are disputed and others are thought to be too easily misinterpreted, too subjective and lacking a sound basis and objective criteria (Lichtenberg et al., 2023, Percival et al., 2015a, Wei et al., 2019, Swanson et al., 2022, Schultz et al., 2017, Percival et al., 2012). Furthermore, little validation work has been done to determine the abilities of clinical signs and symptoms to confirm biofilm presence in chronic wounds and that which has been done is not comprehensive. Of three studies found in the literature, one attempted to correlate six clinical observations against PNA-FISH and CLSM of biopsies taken from 26 wounds as a secondary objective in their study, and a second used scanning electron microscopy to search for biofilms in debridement samples from 11 wounds that appeared to be covered with an adherent gel-like film (visual indicator) (Hurlow et al., 2016, Johani et al., 2017). A third study that set out to statistically determine the predictive abilities of clinical signs and symptoms along with a bacterial fluorescence imaging device and wound blotting against 4mm x 4mm wound scrapings taken from 40 wounds and assessed for biofilm with scanning electron microscopy and quantitative microbiology, did not explicitly assess individual signs and symptoms but rather assessed them as a collective unit (Mayer et al., 2024). The first study concluded that the clinical observations for the most part could not reliably predict presence of biofilm, the second formed a tenuous link between a visual indicator (gel-like film) and presence of biofilm (75% of the debridement samples contained biofilm) and the third demonstrated sensitivity and accuracy values for an unspecified group of clinical signs of less than 50% (Hurlow et al., 2016, Johani et al., 2017, Mayer et al., 2024).

1.5 Thesis rationale

There is a consensus that biofilm is associated with delayed healing in chronic wounds (Schultz et al., 2017, Swanson et al., 2022). and in the previous paragraphs methods of detection available to assist in confirming their presence in chronic wounds as well as strategies and interventions that are reported to be capable of treating them were described. However, controversies and limitations in regard to these detection and treatment strategies were also described, and these limitations form the basis for concerns in terms of the effectiveness of antibiofilm strategies and good antimicrobial stewardship. The concerns are firstly, that if the presence of biofilm in chronic wounds cannot be easily and reliably confirmed, how can it be established that it was there in the first place, secondly, that it has indeed been removed and that antibiofilm strategies are truly effective, and thirdly, that removing biofilm really helps to break the inflammatory cycle and allow the wound to progress to healing. Consequently, one must ask if there is a danger of administering unnecessary treatments that are not risk free to patients and if antimicrobial stewardship principles are being compromised.

A potential solution would be to determine if a reliable means of confirming biofilm in chronic wounds that can be used in a clinical setting can be developed. Clinical signs and symptoms are commonly used in tools to facilitate confirmation of health conditions such as wound infection, pneumonia and Strep throat (Woo and Sibbald, 2009, Patel, 2021, Wagner and Mathiason, 2008, Gardner et al., 2001). These tools are inexpensive and easy to use and if properly developed, they will have statistically proven confirmatory abilities (Beattie and Nelson, 2006).

As discussed, clinical signs and symptoms are commonly recommended and used to confirm biofilm presence in chronic wounds but yet without any definitive efforts to evaluate their predictive abilities. It is proposed then that a validation process be performed on these signs and symptoms to investigate if they can be used as the basis for a tool that could facilitate confirming the presence of biofilm in chronic wounds.

1.6 Thesis aim

The aim of this research project is therefore to open an avenue for researchers to permit an attempt to create a reliable and valid predictive tool that uses clinical signs and symptoms reported in the literature to be indicative of biofilm in chronic wounds that could aid confirmation of biofilm presence in DFU in a clinical setting.

1.7 Thesis methodologies

The methodology for this project will be guided by elements of the Clinical Prediction Rule (CPR) approach which will be described in chapter 3, section 3.2. It will comprise a scoping review of the literature, taking advantage of my evidence synthesis experience to collate a comprehensive list of clinical signs and symptoms reported to be indicative of biofilm in chronic wounds. The resulting clinical sign and symptom list will then be subjected to an electronic Delphi consensus process wherein an international panel of highly experienced wound care clinicians will refine it by determining which of its items are most likely in their opinion to be indicative of biofilm in chronic wounds. The process will draw upon my extensive professional network initiated during my time with Dr. Sibbald and expanded during my time as a research assistant and PhD student with Prof. Gethin. The items on the finalised list of clinical signs and symptoms must then, according to the CPR have their predictive abilities determined statistically. This can be achieved by means of a clinical study in which the wounds of a sample of consenting participants are assessed for presence or absence of each item. The assessments are then compared against gold standard biopsies assessed for biofilm using advanced microscopy techniques.

An access pathway to link a protocol for such a study to an investigative site through the ethical, regulatory and data protection systems will be created, bringing my prior clinical trials experience into play. This pathway will comprise a study protocol, participant information leaflets and consent forms and it will also include a schematic instruction sheet that highlights the institutional offices with whom investigators must engage along with lists of necessary documentation that must be submitted in order to gain regulated access to an investigative site and allow successful completion of the study. An account of my journey through these systems along with associated timelines will also be provided.

Finally, there will be a section on the analysis of clinical samples for biofilm in the laboratory. ESCMID guidelines advise that SEM or CLSM are the most appropriate techniques for detecting biofilm in clinical samples, with the latter often used in conjunction with PNA FISH (Høiby et al., 2015, Fazli et al., 2009, Malone et al., 2017b, Kirketerp-Møller et al., 2008, Johani et al., 2017). However, the PNA-FISH kits commonly by wound care researchers are no longer commercially available and so an alternative fluorescence staining methodology will be presented along with a standard protocol for preparing samples for scanning electron microscopy. This final phase will be facilitated by extensive experience at the laboratory bench that I gained during my MSc degree.

1.8 Thesis chapters overview

Chapter one introduces the student and provides an introduction and overview of the thesis. A rationale and study aim are also provided.

Chapter two provides a review of the literature in which the normal wound healing process will be presented followed by a description of chronic wounds - the consequences of the wound healing process gone wrong. Diabetes mellitus will be discussed prior to introducing the reader to diabetic foot ulceration. Chronic wound infection will be addressed as will biofilms in terms of chronic wounds and strategies for their identification and treatment. This will all lead into the concerns that gave rise to this project and a project rationale.

Chapter three comprises the methods and results sections. The first section describes the methods and findings of the scoping review, and the following section will describe the methods and findings of the electronic Delphi process. A protocol for a clinical study to collect biopsy samples and assess wounds for clinical signs and symptoms of biofilm will be presented followed by an account of a journey through ethical and regulatory systems to gain approved access to an investigative site. The results of this journey – a route map through these systems will then be presented. Finally, there will be a section on sample analysis in the laboratory in which an account of the development of an alternative means of identifying biofilm in biopsy samples to PNA FISH will be provided in addition to preliminary findings and recommendations in terms of the latter.

Chapter four is the discussion chapter This chapter will review the rationale and aims of the work, briefly summarise the results of each phase and then critically discuss them in the context of the literature and in the context of today's clinical research processes in terms of regulatory affairs. Findings will also be critically appraised, lessons learned during the execution of phase methodologies will be presented and observations made while contemplating phase results that further reinforce the importance and significance of the work outlined in this PhD project will be documented.

The conclusion will restate the project rationale, aims and methodological phases. It will then point out what the phases have achieved and contributed to wound care research through working as a unified entity. Finally, this author's hopes for the unified outputs this PhD project will be expressed.

2 Chapter Two: Thesis literature review

2.1 Introduction

The purpose of this literature review is to establish a rationale for this Doctoral project. As a foundational exercise, it will be necessary to provide some basic understanding of a series of concepts and to link them together in a fashion that will give greater meaning and impact to the concerns, rationale and aims of this research. These concepts will progress in a logical manner, introducing the reader firstly to the wound healing process, how it can go awry and give rise to chronic wounds before describing chronic wounds in terms of aetiology, prevalence and impact. The population of interest for this project concerns the diabetic foot ulcer patient and therefore diabetes mellitus must be explored as must the means by which it gives rise to that ulceration and the nature and impact of the ulceration itself. Infection is a grave concern in these wounds and so chronic wound infection will also be described. Finally, the concept of interest for this project is the biofilm bacterial phenotype, and this will be illustrated as will its hypothesised role in chronic infections, treatment and diagnostic modalities and in particular, the issues that surround them.

Once this foundational exercise has been completed and a level of background understanding established, the reader will then be introduced to the rationale, concerns and aim of this research.

2.2 The wound healing process

The skin is the largest and most exposed of the body's organs (Yang et al., 2022). It constitutes approximately 15% of total body weight, and while having sensory, thermoregulatory and maintenance of hydration functions, it is also the body's primary defence, providing protection against toxic substances, ultraviolet (UV) light, mechanical injury and pathogens (Fore, 2006, Yousef et al., 2024, Lawton, 2019).

Upon receiving an injury, the skin immediately initiates a process to restore its integrity and protective functioning through orderly phases of haemostasis, inflammation, proliferation and remodelling/maturation which will be summarised below (Frykberg and Banks, 2015).

2.2.1 Haemostasis

A functioning haemostatic phase requires vascular endothelium, platelets, coagulation factors and their regulators, and a fibrinolytic process working together across primary and secondary haemostatic stages to bring bleeding to a stop (Austin, 2021). Primary haemostasis starts with local vasospasm and vasoconstriction followed by steps of platelet adhesion, activation and aggregation whereby platelets recognise injury and interact with the vascular endothelium to form a weak platelet plug (Austin, 2021, Germann and Stanfield, 2002). During secondary haemostasis the plasma protein fibrinogen is converted into active fibrin by thrombin by the action of a series of plasma protein coagulation factors operating through intrinsic and extrinsic pathways of the coagulation cascade (Germann and Stanfield, 2002). Fibrin molecules form into a stable meshwork of strands or fibrin clot which plugs the damage site in a more permanent fashion (Germann and Stanfield, 2002). Fibrinolysis brings about dissolution of the clot through an enzymatic pathway that incorporates plasmin, a derivative of the plasma protein plasminogen (Austin, 2021).

2.2.2 Inflammation

During the inflammatory phase, mast cells gather and release histamine, prostaglandins, leukotrienes and kinins which stimulate vasodilation, providing an increase in blood flow to the wound site and giving rise to local clinical signs of redness, heat, swelling and pain (Orsted et al., 2017b, Kane, 2007, Ng, 2010). Chemoattractive agents (for example, complement components and transforming growth factor) draw neutrophils to the wound site (Velnar et al., 2009). They pass through endothelial cells, and once in the wound environment they phagocytose and destroy bacteria, foreign material and debris with reactive oxygen species and proteolytic enzymes (Velnar et al., 2009, Schultz et al., 2011). Eventually, through apoptosis, the neutrophil population begins to deplete and monocyte-derived macrophages drawn to the site by chemoattractive agents (for example, clotting factors and cytokines) continue the process of phagocytosis (Velnar et al., 2009). Macrophages ingest and kill bacteria and remove devitalised tissue through the actions of matrix metalloproteinases (MMPs) and elastase, regulating the proteolytic destruction of devitalised wound tissue through secreted protease inhibitors (Schultz et al., 2011). In addition, they mediate transition of the inflammatory phase into the proliferative phase through the release of growth factors and cytokines (for example, platelet-derived growth factor [PDGF], transforming growth factors [TGF] α and β , tissue necrosis factor [TNF] α and fibroblast growth factor [FGF]) (Schultz et al., 2011).

Keratinocytes, fibroblasts and endothelial cells become activated with fibroblasts for example synthesising, depositing and organising new tissue matrix and promoting angiogenesis (Schultz et al., 2011, Velnar et al., 2009). Finally, lymphocytes arrive at the site, drawn in by action of interleukin-1 (IL-1), complement components and immunoglobulin G (IgG) breakdown products (Velnar et al., 2009). The absence of neutrophils and the decline of the number of macrophages in the wound signify that the inflammatory phase is coming to a conclusion (Schultz et al., 2011).

2.2.3 Proliferation

Once haemostasis has been achieved and an appropriate immune response established, the focus of the wound shifts towards repair (Velnar et al., 2009). The proliferative phase of the wound healing process replaces the provisional fibrin matrix or clot with an extracellular matrix of collagen fibres, proteoglycans and fibronectin to restore structure and function of tissue, and promotes angiogenesis to replace damaged capillaries, the formation of granulation tissue, and re-epithelialization (Orsted et al., 2017b, Schultz et al., 2011). Granulation tissue is the intermediary substitute for normal dermis and is a pink, vascular, fibrous tissue which replaces the provisional wound matrix at the wound site (Schultz et al., 2011, Harper et al., 2014). Unlike unwounded tissue, it contains a dense network of capillaries, elevated quantities of fibroblasts and macrophages, randomly arranged collagen fibres (with a larger proportion of type III collagen) and it exhibits a high rate of metabolism which facilitates cellular division and migration, and protein synthesis (Schultz et al., 2011).

Fibroblasts are significant elements in the proliferative phase and those in dermis around the wound are stimulated to proliferate by TGF- β and PDGF released from the haemostatic clot (Harper et al., 2014, Schultz et al., 2011, Velnar et al., 2009, Landén et al., 2016). They migrate into the wound, drawn in a chemotactic response to cytokines and growth factors such as PDGF, TGF- β and basic fibroblast growth factor (bFGF) produced by platelets and macrophages (Landén et al., 2016). They break down ECM material by secreting proteolytic enzymes such as collagenase, gelatinases and stromelysins (MMP 1, 2 & 9, 3) and move through it in the direction of the chemoattractant concentration gradient by extending cytoplasmic projections that bind to matrix components such as fibrin, vitronectin and fibronectin via integrin receptors, pulling themselves from binding site to binding site with their cytoskeleton network of actin fibres (Landén et al., 2016, Schultz et al., 2011). Once in the wound environment they begin to proliferate profusely, secreting proteinases such as MMPs

to degrade the provisional wound matrix and start synthesizing proteoglycans, hyaluronic acid, fibronectin, glycosaminoglycans, elastin and collagen which form granulation tissue (Schultz et al., 2011, Harper et al., 2014, Velnar et al., 2009, Landén et al., 2016). Transforming growth factor- β regulates ECM deposition by promoting increased production of matrix proteins, downregulating secretion of proteases that degrade matrix and stimulating production of tissue inhibitor of metalloproteinases (TIMP) which also prevents matrix degradation (Schultz et al., 2011).

Angiogenesis begins as soon as the haemostatic plug forms and continues throughout all phases of the wound healing process, allowing restoration and regeneration of the blood vessel network in the wound which will provide the nutrients and oxygen necessary for tissue repair (Landén et al., 2016, Harper et al., 2014). The centre of the wound is relatively avascular and its tissue depends on oxygen and nutrients diffusing in from undamaged capillaries at its edges, but a dense network of initially fragile and permeable capillaries derived from healthy vessels quickly begins to form (Harper et al., 2014, Velnar et al., 2009). The process is activated by microenvironmental factors such as low oxygen tension, low pH and high lactate levels, and by angiogenic factors acting on endothelial cells of existing vessels including FGF, vascular endothelial growth factor (VEGF), PDGF, angiogenin, TGF- α & TGF- β , thrombin and bFGF (Landén et al., 2016, Schultz et al., 2011, Harper et al., 2014, Velnar et al., 2009). The anti-angiogenic factors angiostatin, endostatin, thrombospondin and pigment epithelium-derived growth factor (PEDF) regulate the process (Schultz et al., 2011, Velnar et al., 2009). In response to stimulation, vascular endothelial cells begin to secrete proteolytic enzymes which dissolve basal lamina in parent vessels and pass through the ECM (Velnar et al., 2009, Landén et al., 2016). The cells migrate chemotactically towards the source of angiogenic stimulus, moving in a series of coordinated cytoskeletal changes which cause cells to extend protrusions in the direction of movement, adhere to the substratum and generate traction forces which propels trailing cytoplasm forwards (Landén et al., 2016, Velnar et al., 2009, Schultz et al., 2011). These cells then start proliferating to form buds or sprouts and as the tips of these sprouts extend and encounter other sprouts they develop clefts which will form the lumen of the developing vessel (Schultz et al., 2011). The process continues until the capillary system is repaired and the oxygen and nutrient needs of the wound tissue can be met (Schultz et al., 2011). To cover the wound, epithelial cells from the wound edge begin migrating soon after injury, distributing a sheet of cells that is attached to the matrix below in a process mediated by nitric oxide, cytokines and growth factors including epidermal growth factor (EGF), keratinocyte growth factor (KGF), IGF-I, nerve growth factor (NGF) and TGF- α , secreted from multiple cell types

in the wound (Harper et al., 2014, Velnar et al., 2009, Schultz et al., 2011, Landén et al., 2016). Migration is initiated when membrane-associated kinases become activated by loss of contact inhibition and physical tension at cell adhesion structures (that is, desmosomes and hemidesmosomes) increasing permeability for calcium which signals re-organisation of cytoskeleton driving migration (Landén et al., 2016). The proteolytic enzymes collagenase and elastase release the migrating cells from their original sites (Landén et al., 2016). Integrin receptors are expressed and basal epithelial cells flatten and migrate as a monolayer over new granulation tissue, attaching to it and tracking in the direction of collagen fibres (Schultz et al., 2011, Velnar et al., 2009). Epithelial cells in the leading edge release matrix metalloproteinases allowing the advancing cells to penetrate necrotic tissue, scab and eschar (Schultz et al., 2011). As migration proceeds, keratinocytes at the basal layer of the wound edge and epithelial stem cells from hair follicles and sweat glands start to proliferate, generating more cells to cover the wound (Landén et al., 2016, Schultz et al., 2011). Once cells come into contact and a confluent sheet is formed, migration stops and new adhesion structures are formed (Schultz et al., 2011, Landén et al., 2016). The monolayer enters a proliferative mode, and keratinocytes secrete proteins to rebuild the basement membrane and stratified layers of epidermis re-establish themselves, maturing and restoring barrier function (Landén et al., 2016, Schultz et al., 2011, Velnar et al., 2009). Intercellular desmosome and hemidesmosome attachments to newly formed basement membrane are also re-established (Schultz et al., 2011).

2.2.4 Maturation/remodelling

In the final or maturation/remodelling phase of the wound healing process, new epithelium develops, and granulation tissue begins to mature into a scar (Harper et al., 2014, Schultz et al., 2011, Velnar et al., 2009). This phase is tightly controlled by regulatory mechanisms which strive to maintain a balance between synthesis and degradation as collagen and other proteins are laid down in the wound (Velnar et al., 2009, Harper et al., 2014). As the intracellular matrix matures, collagen type III produced initially by fibroblasts is replaced by collagen type I, collagen bundles increase in diameter, resulting in progressive increase in tensile strength and hyaluronic acid and fibronectin are degraded (Velnar et al., 2009, Schultz et al., 2011, Harper et al., 2014). The initial deposition of collagen bundles is random and disorganised, but with time and facilitated by the enzyme lysyl oxidase secreted into the ECM by fibroblasts, new matrix fibres form covalent crosslinks, and they orient themselves along tension lines which increases the tensile strength of the new tissue (Velnar et al., 2009, Schultz et al., 2011, Orsted

et al., 2017b). Tissue at the wound site will never regain the original level of tensile strength prior to injury but typically will reach 50% at three months and 70-80% long term (Harper et al., 2014, Orsted et al., 2017b). The process of synthesising and breaking down of collagen, and that of ECM remodelling is continuous (Velnar et al., 2009). The most significant enzymes are the MMPs (for example, collagenases, gelatinases and stromelysins) produced by fibroblasts, macrophages and neutrophils, and serine proteinases (for example, neutrophil elastase) (Velnar et al., 2009, Schultz et al., 2011). The activity of these proteolytic enzymes is tightly regulated by the inhibitory factors TIMPs, α 1-protease inhibitor (α 1-PI) and α 2 macroglobulin, and as inhibition of MMPs gradually increases, proteolysis decreases allowing new matrix to accumulate (Schultz et al., 2011, Velnar et al., 2009). During the final stages of the remodelling phase, underlying connective tissue contracts through fibroblast/ECM interactions and draws wound margins together in a process regulated by multiple factors including PDGF, TGF- β and FGF (Velnar et al., 2009). As wound healing progresses, the fibroblast and macrophage populations are reduced by apoptosis, vascularity decreases with capillaries aggregating into larger vessels, general metabolic activity in the wound site diminishes and the scar changes from red to pink and finally to grey (Velnar et al., 2009, Harper et al., 2014, Orsted et al., 2017b, Schultz et al., 2011). The final result is high-tensile strength mature scar with reduced numbers of cells and blood vessels (Velnar et al., 2009).

2.2.5 Exceptional circumstances

Under normal circumstances these phases proceed in an overlapping but orderly fashion until wound closure is achieved - typically within four to six weeks, but in certain situations, usually in cases where there are underlying chronic comorbidities, the process breaks down and the wound becomes chronic and slow to heal, trapped in an ongoing inflammatory feedback loop (Frykberg and Banks, 2015, Wallace et al., 2024, Wolcott et al., 2008, Menke et al., 2007). Such a state creates a toxic environment in the wound that favours degradation, with excessive numbers of inflammatory cells, abnormally high levels of MMPs and inflammatory cytokines but insufficient quantities of MMP inhibitors and factors that promote proliferation (Verdolino et al., 2021, Menke et al., 2007).

The population of interest for this doctoral work comprises a group of individuals who exemplify the consequences of a wound healing process gone awry, suffering from chronic wounds secondary to diabetes mellitus or diabetic foot disease (DFD).

The reader will next be provided with some understanding of this condition, context will be created, and its origins and development will be illustrated. Context will firstly be set by discussing chronic wounds in terms of definitions, the aetiologies and pathophysiologies commonly encountered in our clinics, and their prevalence and impact. An overview of diabetes will then be provided with a focus mostly on diabetes types 1 and 2. The means by which hyperglycaemia associated with diabetes mellitus causes damage to tissues in the body will then be presented followed by a description of the pathophysiology, prevalence and impact of diabetic foot ulceration.

2.3 Chronic wounds

2.3.1 Definition of chronic wounds

Chronic wounds have variously been defined as ‘wounds that fail to proceed through the normal phases of wound healing in an orderly and timely manner’, ‘wounds that have not proceeded through an orderly and timely reparation to produce anatomic and functional integrity after three months’ or ‘wounds that lack a 20-40% reduction in size after two to four weeks of optimal treatment or when there is not complete healing after six weeks’ (Ivory et al., 2021). Definitions may vary, but the common denominator that separates acute wounds from chronic would appear to be the rate of healing (Menke et al., 2007, Ivory et al., 2021).

2.3.2 Aetiologies & pathophysiologies of chronic wounds

Susceptibility to chronic wounds often lies in faulty physiological processes resulting from a predisposing comorbidity and commonly observed and treated wound aetiologies include those secondary to diabetic, venous, arterial and pressure-related conditions (Bowler et al., 2001, Bowers and Franco, 2020).

Venous dysfunction or insufficiency stems from venous hypertension which damages valves in the veins, causing reflux and reduced venous return which lead to blood pooling in the lower limbs, inflammation and hypoxia (Krizanova et al., 2023). The constant excessive pressure in the veins causes capillary dilation, deposition of blood in skin microvasculature, hypervolemia and peripheral oedema (Krizanova et al., 2023). Normal exchange of blood between venous and arterial capillaries no longer occurs and the buildup of deoxygenated venous blood causes hypoxia and venous ischaemia (Krizanova et al., 2023). Consequently, injuries to the skin in

the lower limbs may fail to heal and degrade into venous leg ulcers (VLU) (Institute for Quality and Efficiency in Health Care (IQWiG), 2006).

Arterial insufficiency or peripheral arterial disease is a progressive disorder of the aorta and lower limb arteries brought about by atherosclerosis, where the arterial lumen gradually becomes blocked with plaques composed of macrophages and cholesterol (Rabbia, 2012, Zemaitis et al., 2024). These plaques or atheromas form between the endothelium and the smooth muscle layer of the artery (Rabbia, 2012). Initially, arteries are able to compensate for the occlusion by dilating but, this will eventually reach an end point and the plaque will narrow the lumen and restrict blood flow (Zemaitis et al., 2024). Affected arteries may also exhibit calcification and fibrosis which causes them to harden (Rabbia, 2012). Arterial ulcers in the lower extremities are the result of tissue loss brought about by impaired blood flow (Zemaitis et al., 2024).

Pressure ulcers (PU) or pressure injuries do not necessarily have involvement of intrinsic comorbidities and arise as a result of sustained external pressure to the skin, mainly but not always in the areas of the sacrum, buttocks and heels, but underlying pathologies can contribute to their chronicity (Bowler et al., 2001). Diabetic foot ulceration will be described in section 2.5.

2.3.3 Prevalence and impact of chronic wounds

In terms of prevalence, nonhealing wounds are estimated to affect 1-2% of the population in developed countries and as the population ages, the number of chronic wounds is expected to increase significantly (Maheshwari, 2024). A meta-analysis of three studies from 2019 estimated that chronic wounds of mixed aetiologies had a pooled prevalence of 2.21 per 1000 population, and a second meta-analysis from the same year estimated the pooled prevalence of chronic leg ulcers to be 1.5 per 1000 population (Martinengo et al., 2019, Ivory et al., 2021). A cross-sectional survey conducted on 10,144 patients from 36 hospitals across 18 National Health Service (NHS) trusts in the United Kingdom recorded an overall prevalence of PU (that is, the proportion of patients with 1 or more PU) of 9.04% (95% CI 8.48%-9.60%) (Stephenson et al., 2021). The number of persons with diabetes is expected to reach more than 400 million by 2025 and one quarter of these will suffer foot ulceration in their lifetime (Maheshwari, 2024).

Regardless of aetiology, chronic wounds are burdensome to the individual and to health care systems (Ivory et al., 2021). A survey incorporating the Cardiff Wound Impact Schedule of 113

persons with chronic wounds from Australia and Wales (Kapp and Santamaria, 2017), found global health-related quality of life (HRQoL) and satisfaction with quality of life to be suboptimal with scores of six out of ten for both measures. Quality of life scores for physical symptoms and daily living components were described as reasonable (64/100 on average) and social life scored 72/100, but the wellbeing component only scored 40/100. A systematic review from 2018 that included 19 HRQoL studies with a total of 1,867 patients and using 9 different measurement instruments including the 36-item Short Form survey (SF-36), Euroqol-5D (EQ-5D) and World Health Organisation quality of life-BREF (WHOQOL-bref) (Olsson et al., 2019), reported lower HRQoL scores for physical functioning-related/mobility domains. Four of the included studies showed lower scores in emotional and mental health domains while five showed no statistical difference for same when compared to controls. Four studies with controls demonstrated significantly lower scores for patients with chronic wounds in terms of bodily pain and pain/discomfort. Patients with chronic wounds were found to spend an average of 10.44% (standard deviation [SD] 16.47%) of their disposable income on wound products and they also incurred costs in terms of appointments, travel, lost wages, communication, additional treatment/equipment, and clothing (Kapp and Santamaria, 2017). The annual cost of chronic wounds (DFU, VLU and PU) to the NHS was estimated to be approximately £5 billion (at 2017-2018 prices) which translates to an average of £4,645 per patient, while in Germany, primary and outpatient costs for leg ulcers ranged from US\$449 to US\$2,307 per patient annually, and from US\$13,334 to US\$13,761 to society (Guest et al., 2020, Olsson et al., 2019). Here in Ireland, a decision analytic model incorporating a bottom-up, prevalence-based analysis published in 2019 estimated the annual cost of wound care to our health care system to be almost €830 million (Gillespie et al., 2019). This accounts for 5% of the total Irish public health expenditure and equates to €3,941 spent per patient.

2.4 Diabetes mellitus

2.4.1 An overview of Diabetes mellitus

Diabetes mellitus (DM) or simply diabetes is a non-communicable, complex metabolic disorder that manifests as hyperglycaemia - inappropriately elevated glucose levels in the blood that affects one in 11 adults worldwide (Gupta et al., 2024, Banday et al., 2020, Sapra and Bhandari, 2025). Under normal circumstances, glucagon produced by α cells and insulin produced by β cells in the islets of Langerhans regions of the pancreas work in opposition to each other to regulate sugar levels in the blood (Sapra and Bhandari, 2025). Glucagon comes

into play when blood glucose levels are low, stimulating hepatic glucose production through glycogenolysis and gluconeogenesis, whereas insulin enables uptake of glucose from the blood to be used as a source of energy in the body (Gupta et al., 2024, Hædersdal et al., 2023). However, this process can become disrupted or impaired and diabetes arises from anomalies with respect to insulin secretion, insulin action or both (Banday et al., 2020). There are several variations of the disorder and it can be classified into type 1 or type 2 diabetes, maturity onset diabetes of the young (MODY), gestational diabetes, neonatal diabetes and diabetes secondary to conditions such as acromegaly, Cushing's disease, pheochromocytoma, chronic pancreatitis and malignancy, or secondary to use of medications such as phenytoin or glucocorticoids (Banday et al., 2020, Sapra and Bhandari, 2025, Gupta et al., 2024). For the purposes of this literature review however, consideration will only be given to diabetes types 1 and 2.

2.4.2 *Diabetes mellitus Type 1*

Type 1 diabetes mellitus (T1DM), also known as insulin-dependent diabetes mellitus (IDDM) or juvenile diabetes contributes to approximately 5–10 % of all cases and presents mostly in children or adolescents (Banday et al., 2020, Sapra and Bhandari, 2025). T1DM is an autoimmune disorder in which T-cells of the immune system attack and destroy insulin producing β cells leading to a deficiency or complete absence of insulin in the body and ultimately hyperglycaemia (Sapra and Bhandari, 2025). In most cases (juvenile onset in children and adolescents) the rate of development of T1DM is rapid with β cell destruction and failure occurring quickly, leading to diabetic ketoacidosis (DKA) which is often described as the first manifestation of the disease (Banday et al., 2020). In some cases though it can develop more gradually (late onset in adults), with mild increases in fasting blood glucose levels which will only degrade into severe hyperglycaemia (with or without ketoacidosis) in the presence of physiological stressors such as serious infection or onset of other disorders (Banday et al., 2020). Regardless of the rate of progression, affected individuals will become either severely or absolutely deficient in insulin and dependent on insulin treatment (Banday et al., 2020). Several immune markers, that is autoantibodies are linked to β cell destruction in T1DM, and these include among others, glutamic acid decarboxylase autoantibodies (GADAs), islet cell autoantibodies (ICAs) to β cell cytoplasmic proteins, autoantibodies to the tyrosine phosphatases and insulin autoantibodies (IAAs) (Banday et al., 2020). The glutamic acid decarboxylase 65 (GAD 65) autoantibody is found at time of diagnosis in 80% of all individuals

with T1DM followed by ICAs in 69 – 90% of these individuals, and the IAAs are important markers that present in infants and young children (Banday et al., 2020).

There is a genetic component to the origins of T1DM; its autoimmune nature is associated with human leucocyte antigen (HLA), its links to DQA and DQB genes and influence from DRB genes, all of which are involved with the immune response (Banday et al., 2020). Individuals having HLA and HLA DQ genotypes are at increased risk of developing two or more autoantibodies that lead to β cell destruction (Gupta et al., 2024).

In terms of epidemiology, T1DM has a prevalence of approximately 2.3/1000 in persons under 20 years (Sapra and Bhandari, 2025). Onset increases from birth, peaking firstly at 4 – 6 years and again at 10 – 14 years, and approximately 45% of children present with T1DM before 10 years of age (Sapra and Bhandari, 2025). Worldwide, incidence of T1DM is on the rise with rates in Europe, Australia and the Middle East increasing by 2 – 5% yearly, and those in the United States of America (USA) increasing in most ethnic groups by 2% each year (Sapra and Bhandari, 2025). According to Diabetes Ireland, there were 20,081 prevalent cases of T1DM in the country in 2016 and 17,053 of these were adults over 18 years (Diabetes Ireland, 2022).

2.4.3 *Diabetes mellitus Type 2*

Type 2 diabetes mellitus (T2DM), alternatively non-insulin dependent diabetes (NIDDM) or adult onset diabetes makes up 90-95% of all cases and presents usually in middle-aged to older adults (Sapra and Bhandari, 2025, Gupta et al., 2024, Banday et al., 2020). This condition arises when the body develops a functional deficit of insulin through resistance and β cell dysfunction (Banday et al., 2020, Sapra and Bhandari, 2025). Excess fatty acids and proinflammatory cytokines disrupt biochemical pathways in cells of adipose, muscle and liver tissues, resulting in impaired glucose transport and increased fat breakdown which leads to an inadequate response to or insensitivity of these tissues to insulin (Sapra and Bhandari, 2025, Banday et al., 2020). As a result of this acquired insulin insensitivity, β cells become triggered into a hyper functional state to compensate and maintain normoglycaemia, and although the higher levels of circulating insulin initially prevent hyperglycaemia, this state cannot be maintained over the long term and β cell functioning will start to decline leaving an inevitable hyperglycaemic state (Banday et al., 2020). Additionally, the body responds to the insulin imbalance by increasing glucagon production which further exacerbates the hyperglycaemic state (Sapra and Bhandari, 2025).

Unlike T1DM, onset of T2DM is more insidious and the condition often develops slowly over years; sometimes there can be mild hyperglycaemia but without any apparent symptoms, and as such it may remain undiagnosed until symptoms of weight loss, impaired vision, impaired growth, polyuria and polydipsia associated with severe hyperglycaemia in the advanced stages of the disorder become manifest (Sapra and Bhandari, 2025, Banday et al., 2020).

Despite being diminished in T2DM, insulin levels are still adequate to stave off DKA and in the absence of severely stressful pathophysiological conditions such as infection, it will not likely occur and thus patients with T2DM may not require insulin therapy either at time of disease onset or throughout their lifetime (Banday et al., 2020).

The origins of T2DM are complex and typically comprise an interplay between genetic and environmental/lifestyle factors (Banday et al., 2020, Sapra and Bhandari, 2025, Gupta et al., 2024). Environmental/lifestyle factors include increasing age, obesity, family history of diabetes, physical inactivity/sedentary lifestyle, alcohol and nicotine consumption, prior gestational diabetes in women, ethnicity and pathophysiological conditions such as hypertension, metabolic syndrome and dyslipidaemia (Gupta et al., 2024, Banday et al., 2020). Obesity is significant because it affects tissue sensitivity to insulin and most patients with T2DM are thus overweight or obese (Banday et al., 2020). Furthermore, distribution of fat in the body is important with increased abdominal visceral fat showing a stronger association with development of the disease more so than increased gluteal, subcutaneous or peripheral fat (Banday et al., 2020).

In terms of genetics, there appears to be no association between T2DM and genes involved in the immune response (Banday et al., 2020). However approximately 50 polymorphisms that encode for proteins involved in processes such as pancreatic development, insulin synthesis and resistance, deposition of amyloid protein in β cells and impaired regulation of gluconeogenesis have been identified that may contribute to either risk of development of T2DM or protection from it (Sapra and Bhandari, 2025). The hereditary profile of T2DM is thought to be stronger than that of T1DM (Sapra and Bhandari, 2025). A family history of DM accounts for 25% of all cases, there is a strong association with first-degree blood relatives (the majority of T2DM patients have at least one parent with the disease) and in the case of monozygotic twins with one affected twin, the likelihood of the other developing T2DM in their lifetime is 90% (Sapra and Bhandari, 2025, Gupta et al., 2024, Banday et al., 2020). In addition, the disorder tends to occur more often in persons coming from Native, African and Asian American ethnic backgrounds, and Hispanic and Latino backgrounds (Banday et al., 2020).

From an epidemiological perspective, the global prevalence of T2DM in 2017 was estimated to be 6.38% (6059/100,000) with 4.4% of those aged 15 – 49 years, 15% aged 50 – 69 and 22% aged over 70 years (Khan et al., 2020). Incidence was found to peak at 55 years of age and global prevalence is expected to increase to 7079/100,000 cases by 2030 (Khan et al., 2020). Here in Ireland, 10% of adults aged 50 years and over have T2DM and this rises to 16% in those aged 80 and over (Diabetes Ireland, 2022).

2.4.4 Hyperglycaemia and its complications

Hyperglycaemia attributable to diabetes and associated dysfunction in carbohydrate, fat and protein metabolism will disrupt the normal functioning of multiple organs in the body and cause them serious damage (Banday et al., 2020). Chronic hyperglycaemia causes glucose to bond covalently with plasma proteins in a nonenzymatic process called glycation, forming advanced glycation end products (AGEs) which have alterations in their molecular conformation, disrupted enzymatic functioning and impaired receptor functioning (Singh et al., 2014).

The exact process of glycation is not fully understood, but it adversely affects the micro- and macro-vasculature fundamental to the structure and functioning of organs throughout the body (Sapra and Bhandari, 2025, Banday et al., 2020, Singh et al., 2014). Especially affected are the eyes, where damage to blood vessels leads to retinopathy and blindness, the kidneys, with damage leading to nephropathy and end-stage renal disease (ESRD), and the cardiovascular system, where blood vessel endothelial tissue is adversely affected and hyperlipidaemia and increased platelet activity and viscosity present, eventually bringing about atherosclerotic cardiovascular disease (ASCVD) which includes complications such as hypertension, peripheral arterial disease (PAD), stroke and myocardial infarction (Singh et al., 2014, Banday et al., 2020, Sapra and Bhandari, 2025, Bandyk, 2018).

The nervous system is also affected by the process of glycation with damage to the autonomic system leading to cardiovascular, gastrointestinal and genitourinary dysfunction, and damage to the peripheral system which, as shall be illustrated in section 2.5, in conjunction with PAD contributes greatly to the development of lower extremity ulcerations, infections and amputations (Banday et al., 2020).

In these last two sections, chronic wounds and diabetes mellitus were introduced. The definition of what constitutes a chronic wound was considered and some of the common

chronic wound types were presented in terms of aetiology, pathophysiology, prevalence and impact. Diabetes mellitus and the insulin dysfunction that underlies the condition were also briefly explored before an examination of that dysfunction in terms of the more commonly encountered types 1 and 2 diabetes subtypes. Finally, an overview of how insulin dysfunction brings about tissue damage in the body was provided.

Now, in the hope that an adequate background understanding has been presented, the next section will proceed to describe one of the pathologies resulting from hyperglycaemia in more detail - that of DFU, the pathology of concern in this Doctoral work.

2.5 Diabetic foot ulceration

The International Working Group on the Diabetic Foot (IWGDF) define the diabetic foot as ‘infection, ulceration or destruction of tissues of the foot of a person with current/previously diagnosed diabetes mellitus, usually accompanied by neuropathy and/or peripheral arterial disease in the lower extremity’ (van Netten et al., 2020).

The pathophysiology of chronic wounds secondary to diabetes mellitus has been described as a triad of neuropathy, arterial occlusion and trauma with secondary infection (Bandyk, 2018, Raja et al., 2023). Structural deformities also feature and the at-risk foot will likely break down when combinations of these factors present (Rathur and Boulton, 2007, Pataky and Vischer, 2007).

Diabetes-related neuropathy is responsible for 45 to 60% of cases of ulceration (Amin and Doupis, 2016). It causes both loss of protective sensation and poor balance or unsteadiness, and because of motor fibre deterioration, muscles become atrophied, tendons and ligaments lose elasticity and they stiffen and contract, flexor/extensor function in the foot reduces and joint stability is lost (Amin and Doupis, 2016, Boulton et al., 2018, Ahmad, 2016). Deformities such as clawing or hammering of the toes and raised metatarsal heads manifest and zones of high pressure develop on the sole of the foot at the metatarsal heads that are vulnerable to trauma from walking (Bandyk, 2018, Ahmad, 2016). Walking, sensation loss and repeated trauma to the foot causes protective plantar fat pads to atrophy and dislocate, putting the foot in even more danger of skin breakdown, and if not noticed and treated promptly, ulceration and infection can ensue (Bandyk, 2018, Raja et al., 2023).

Furthermore, autonomic dysfunction impairs production of sweat, rendering the skin of the foot susceptible to cracks and fissures from dehydration which provide points of entry for pathogens (Raja et al., 2023, Bandyk, 2018).

Peripheral arterial disease is estimated to be present in 50–60% of patients with DFU, affecting both large and small vessels of the extremities (Boulton et al., 2018, Ahmad, 2016). Hyperlipidaemia, hyperglycaemia, hypertension and smoking are all significant contributors, causing pressure in the micro-vessels to increase which results in damage to endothelial tissue, sclerosis and diminished capillary capacity (Ahmad, 2016, Boulton et al., 2018). In addition, calcium deposits and builds up in the intima and media of the arteries making them rigid and incompressible, and the foot eventually becomes so poorly perfused that tissue integrity cannot be adequately maintained (Bandyk, 2018, Ahmad, 2016).

This damaged and ill-perfused tissue also allows for easy penetration to fascia by bacteria with the resulting infections contributing even further to delayed healing and morbidity (Bandyk, 2018, Raja et al., 2023).

Of those diseases that most impact the individual and society on a global scale, DFD takes 10th place (Bus et al., 2020). On an individual level, the adverse impact of DFU on HR-QoL has been well studied and documented (Haryanto et al., 2023, Mairghani et al., 2024, Polikandrioti et al., 2020, Khunkaew et al., 2019), and the economic burden is significant with DFU costing 9 – 13 billion United States dollars (USD) on top of existing diabetes-related costs in North America, and £1.3 billion annually in the United Kingdom (UK) (Jodheea-Jutton et al., 2022, Guest et al., 2020)

According to McDermott et al., prevalence estimates for diabetic foot ulceration vary depending on the approach to surveillance, the completeness of follow-up and how diabetes and ulceration are defined (McDermott et al., 2023). They cite the International Diabetes Federation which estimates that 40 to 60 million people are affected by DFU (2015 estimates ranged from 9 to 26 million), and a meta-analysis from 2017 that calculated a 6.3% prevalence of DFU among adults with diabetes or 33 million people worldwide. (International Diabetes Federation, 2020, McDermott et al., 2023, Zhang et al., 2017). In the UK, the annual prevalence of DFU among its adult population in 2017/2018 was calculated by Guest et al. to be 0.0063 (Guest et al., 2020). A review by Armstrong et al. from 2023 reports that approximately 18.6 million people with diabetes develop a foot ulcer each year (Armstrong et al., 2023, Zhang et al., 2017). Prevalence of ulceration is lower in adults with T1DM than in those with T2DM and this may be due to factors such as younger age of patients with T1DM and cumulative duration of diabetes, study design differences, differing data collection methodologies, and a lack of representative cohorts of people with T1DM (McDermott et al., 2023).

The lifetime risk of ulceration is reported to be in the range of 12-25% with increases in estimated life expectancy potentially pushing these figures as high as 19–34% (McDermott et

al., 2023, Armstrong et al., 2017). Healing rates for DFU can be protracted and infection, lower extremity amputation and mortality are commonly associated complications (McDermott et al., 2023, Boulton et al., 2018, Hobizal and Wukich, 2012).

The lifetime risk of lower extremity amputation in people with DFU is at least 19% and it occurs in the later stages of diabetes with infection thought to be a commonly occurring causative factor, indeed 15 – 20% of moderate to severe infections in diabetic foot ulceration ultimately result in lower extremity amputation (McDermott et al., 2023, Boulton et al., 2018, Armstrong et al., 2023). Mortality in people with DFU has been estimated through meta-analysis to be 13.1% at one year and 49.1% at 5 years, and cardiovascular disease and infection are reported to be leading causes (McDermott et al., 2023, Chen et al., 2023). Furthermore, lower extremity amputations which are frequently a result of infection, increase the 5-year mortality rate to beyond 70% (Armstrong et al., 2023).

Infection is thus a grave complication, bringing with it increased risk of morbidity and poor clinical outcomes (Pickwell et al., 2015, Ivory et al., 2018, Hobizal and Wukich, 2012, McDermott et al., 2023). Recurrent wounds, long-term wounds and wounds that probe to bone have a higher risk of infection and relapsed or recalcitrant infections are common with up to 25% of DFI persisting after 10 to 20 days despite debridement (McDermott et al., 2023). Foot infection is one of the most common diabetes-related causes of hospitalization in the US, accounting for 20% of all admissions and 10-45% of patients hospitalised for DFI will require readmission within a year (McDermott et al., 2023, Hobizal and Wukich, 2012). Patients with an infected DFU run a 50-fold increased risk of hospitalization and a 150-fold increased risk of lower extremity amputation (LEA) versus patients with a non-infected DFU, and 1 in 6 patients with a DFI will die within a year (Ivory et al., 2018, Pickwell et al., 2015, Hobizal and Wukich, 2012).

Now that diabetic foot ulceration - the condition of interest for this work has been defined and pathophysiology and epidemiology discussed, in particular illustrating the significance and gravity infection carry in relation to these wounds, wound-related infection will be considered. Wound infection and factors that determine host susceptibility will be defined. A distinction between acute and chronic infection will be made and the conceptual continuum of progression of the latter will be described including the clinical manifestations that appear along the way. Biofilm, the concept of interest for this work will also be introduced and discussed in the context of chronic wound infection before a key examination of the strategies used for its

treatment and diagnosis, especially that of clinical signs and symptoms to confirm its presence is undertaken.

2.6 Wound infection

Wound infection can be defined as a ‘host inflammatory response to interfering microorganisms that either directly or indirectly damages host tissue, hence preventing wound healing’ (Hurlow and Bowler, 2022). Whether this interference leads to wound infection and adverse effects on the individual, and if so to what degree, is determined by the resilience of the host/immune system, the microbial load and species, and specific species combinations plus the degree of functional synergy that operates between them (Hurlow and Bowler, 2022, Swanson et al., 2022). In an acute scenario, infection manifests as an obvious or overt condition where invading bacteria are handled effectively and rapidly by the innate immune system and antibiotic therapy where necessary (Hurlow and Bowler, 2022). Chronic wounds on the other hand are susceptible to infections which can result in delayed healing, negative impact on quality of life, significant patient morbidity and mortality, multiple health service visits and increased hospital admission (Siddiqui and Bernstein, 2010, Swanson et al., 2022). In a chronic scenario, infection operates on a continuum of conceptual stages described as follows that manifest sequentially as the microbial load in a wound increases (Swanson et al., 2022). In the first or contamination stage, there are microorganisms in the wound, but they are not multiplying and there is no reaction from the host, neither is there any clinically observable delay in healing. The continuum then moves to a stage of colonisation where the microorganisms begin to multiply in a limited fashion but exhibit low virulence. Again, there is no significant host reaction or observable delay in healing (Swanson et al., 2022). As the colonising microorganisms continue to proliferate, a host response will sooner or later present characterised by covert or subtle signs of local infection including hypergranulation, bleeding and friable granulation, pocketing in granulation tissue and epithelial bridging, increased amounts of exudate and delayed healing (Swanson et al., 2022). Progression of this local infection will manifest classic cardinal or overt signs and symptoms including erythema, warmth at the site, swelling, purulent discharge wound breakdown and increase in size, and increasing pain and malodour (Swanson et al., 2022). The infection may then spread to surrounding tissue causing cellulitis, and the degree of microbial proliferation is such that signs and symptoms beyond the edge of the wound can be observed including extending induration, swelling of lymph glands, crepitus, wound breakdown or dehiscence sometimes with satellite

lesions appearing and spreading inflammation or erythema extending more than 2cm from the wound edge (Swanson et al., 2022). Lastly, local infection may develop into systemic infection with microorganisms spreading through the host via vascular and lymphatic systems. The host response affects the whole body and signs and symptoms can include malaise, lethargy/general deterioration, loss of appetite, fever, sepsis, septic shock, organ failure and death (Swanson et al., 2022).

2.7 Biofilms

Bacteria are commonly conceptualised in the planktonic phenotype, that is, single cells suspended freely in a surrounding medium, but they also have an aggregate or community phenotype known as a biofilm and these biofilms can be found in dental plaque, industrial and potable water systems, rivers and streams, soil, public health environments, implantable devices, and living tissue (Donlan, 2002, Percival et al., 2015a, Khatoon et al., 2018, Hurlow et al., 2015).

Clumps of bacterial cells were first observed by Antonie van Leeuwenhoek in the 1650s, subsequently, in 1883, a report emerged of bacterial cells growing on a surface to form clumps and a theory that these clumps or biofilms predominate in nature emerged in 1978 (Lichtenberg et al., 2023, Donlan and Costerton, 2002). The theory proposed that the majority of bacteria (more than 99.99%) exist as sessile, matrix-enclosed biofilms adhering to surfaces in nutrient-adequate aqueous ecosystems, and that these biofilm phenotypes were significantly different from their planktonic counterparts (Donlan and Costerton, 2002). The term ‘biofilm’ referring to these aggregations emerged in 1981 (Lichtenberg et al., 2023, Bjarnsholt et al., 2013).

Morphologically, biofilms are three-dimensional communities of microbial cells existing in close proximity to each other that are encased in a self-produced extracellular polymeric substance (EPS) or extracellular matrix (ECM) of polysaccharides, proteins and glycoproteins that typically but not always adhere to a biotic or abiotic surface (Hurlow et al., 2015, Metcalf et al., 2014, Bjarnsholt et al., 2013, Lichtenberg et al., 2023, Percival et al., 2015a, Yin et al., 2019). ‘The surface does not have to be solid; it can be an attachment of microbes between two immiscible liquids or of microorganisms at a gas-liquid surface’ (Percival et al., 2015a). These basic characteristics are fluid in nature and can, depending on the microbial species and the environment in which they exist differ widely, leading to a large variety of biofilm structures and sizes ranging from large multicellular aggregates to clusters of a just a few micrometres in diameter (Lichtenberg et al., 2023). The biofilm structure allows the microbes within to dictate

their own environment and protect themselves from adverse environmental challenges such as extremes of temperature, pH, dearth of nutrition, host immune defences and antibiotic treatments (Percival et al., 2015a, Yin et al., 2019).

Much of our understanding of biofilms in terms of their physiology and micro-ecology has been derived from *in vitro* work and classically, the biofilm life cycle has been described as a staged process of reversible attachment, irreversible attachment, maturation, and disruption/dispersion (McBain, 2009, Sauer et al., 2022, Crivello et al., 2023). Within this cycle, planktonic cells make contact with a surface and attach through non-covalent, transient interactions (Hage et al., 2022, Crivello et al., 2023). These interactions subsequently develop into stronger, irreversible adhesions facilitated by structures such as pili and flagella, and once stable, surface colonization initiates with formation of an EPS, bacterial division and multiplication, and recruitment of other planktonic cells (Hage et al., 2022, Crivello et al., 2023). Bacterial cells start forming microcolonies, communicating with each other through quorum sensing (regulation of gene expression based on changes in cell population mediated by autoinducer molecules secreted into the extracellular space) and expressing genes for EPS production (Crivello et al., 2023, Preda and Săndulescu, 2019, Sentenac et al., 2021). The microcolony aggregates then become more substantial and a microenvironment forms within, demonstrating pH, oxygen and nutrient gradients which are reflected in the metabolic diversity of cells within the community (Crivello et al., 2023, Shree et al., 2023, Morales and Kolter, 2014). Finally, portions of EPS containing sessile cells detach from the mature aggregate through forces of mechanical stress and shear, and stressors such as high rates of proliferation or variation in oxygen or nitric oxide levels cause cells in the aggregate to revert to the planktonic phenotype and disperse to find and colonize new surfaces (Hage et al., 2022, Crivello et al., 2023).

Biofilms are an essential component of many ecosystems on our planet and are critical for their functioning and maintenance (Sentenac et al. 2021). They help to stabilise friable soils and maintain soil fertility by fixing atmospheric nitrogen and making nutrients available to plant and other soil-dependent organisms for example, and in freshwater streams they facilitate cycling of carbon and other nutrients (Sentenac et al., 2021, Baxter et al., 2026). In addition, they form the basis for many food webs and are consumed by both invertebrates and vertebrates (Sentenac et al., 2021).

Biofilms are also critical for healthy functioning of the human body (Baxter et al., 2026). Commensal biofilms help to maintain health by facilitating immunity, defence against pathogens and nutrient absorption (Sentenac et al., 2021, Baxter et al., 2026). They train the

immune system to discriminate between beneficial and harmful microbes, and they augment its functioning by synthesising antimicrobial agents, competing for resources with pathogens and preventing their establishment in the body (Baxter et al., 2026). In the gut they protect its lining, break down complex carbohydrates and produce vital metabolites, and in the genitourinary tract they act as physical barriers that prevent adherence of pathogenic organisms (Sentenac et al., 2021, Baxter et al., 2026).

However, in order for relationships between host (the human body) and biofilms to remain commensal, a fine balance must be maintained and when health conditions such as cystic fibrosis, immunodeficiencies or diabetes present, or in cases of acute and chronic infections, this balance can become disrupted and once beneficial biofilms can turn pathogenic (Baxter et al., 2024). These now pathogenic biofilms manifest as chronic infections in the lungs, bone and deep tissues. Additionally, they can bring about disease in other ways than by infection. For example, some structural fibres of biofilms in the gut resemble human amyloids and these can trigger immune responses linked to inflammatory bowel disease and neurodegenerative diseases (Baxter et al., 2024). Biofilms are therefore a significant source of concern in the medical fields (Philipp et al., 2024).

The following section will briefly present on the pathogenic role played by biofilms in chronic wounds.

2.8 Biofilms and chronic wound infection

Hurlow and Bowler state that chronic wound infection usually presents as ‘an unclear and prolonged (covert) condition in which biofilm is the root of the problem’ (Hurlow and Bowler, 2022). Indeed, while James et al. estimated biofilms to be present in 6% of 16 patients with acute wounds, they found them to be present in 60% of 50 patients with chronic wounds (James et al., 2008). Malone et al. subsequently estimated the prevalence of biofilm in chronic wounds to be 78% through a systematic review and meta-analysis of the literature, and Attinger and Wolcott and Goswami et al. have reported biofilm to be present in 90 and 100% of chronic wounds respectively, but these latter two estimates are uncited (Malone et al., 2017a, Attinger and Wolcott, 2012, Goswami et al., 2023).

To help conceptualise biofilms more realistically than the somewhat limited, *in vitro*-derived understanding (Vyas et al., 2022, Seth et al., 2012) referred to in section 2.7, and to capture the complex nature of their relationship with the host in the context of a chronic wound infection, Kirketerp-Møller et al. have proposed the Zone model. This model delineates five zones that

radiate outward from bacterial cells in the biofilm (Kirketerp-Moller et al., 2020). Zone one forms the microenvironment adjacent to the individual cells in the biofilm and predicts their phenotype (metabolically active or dormant) depending on where in the biofilm they are located (superficially or centrally) and levels of oxygen, nutrients and minerals. Zone two is the biofilm itself consisting of multiple bacteria in a self-produced matrix plus host DNA, proteins and polysaccharides. Zone three refers to the environment around the aggregate or exobiofilm zone and this is the area where the host reaction is delivered as is the biofilm's defence processes. Zone four constitutes the host tissue around the biofilm, and this is where the wound healing processes take place. This zone is where predisposing host factors and the systemic impact of the conflicting host and bacteria are reflected. Zone four may be different for different biofilms in a wound in that density of bacterial aggregates can be higher in superficial layers of the wound but more diffuse in the deeper layers. Finally, zone five is the host and any predisposing factors such as chronic disease, peripheral arterial disease, neuropathy, a compromised immune system, lower limb oedema etc. The conditions in any zone influence those in another in a complex and dynamic interplay (Kirketerp-Moller et al., 2020). As an example, the authors cite debridement and propose that it induces changes in the zone four environment which can radiate out to all other zones and eventually force bacteria in zone one to change phenotype. Furthermore, passage of oxygen into zone four is regulated to keep host tissue oxygenated, while in zone three host polymorphonuclear leucocytes (PMNs) fight against the biofilm with oxidative bursts that use up oxygen and decrease the levels available for zone two. Host ischaemia (zone five) will further exacerbate the dearth of oxygen available for zone two (Kirketerp-Moller et al., 2020).

Biofilm infections are rarely resolved by the host immune system and they do not respond completely to antibiotic therapy; symptoms caused by planktonic cells released from the biofilm are reversed, but the biofilm itself will remain unaffected (Wolcott et al., 2008, Costerton et al., 1999). Factors contributing to the refractory nature of biofilm infections include species diversity within the biofilm, variability of the infectious environment and the upregulation of virulence and resistance characteristics (Baxter et al., 2026). Furthermore, inability of drugs to penetrate into the biofilm, the presence of dormant or persister cells and enhanced horizontal gene transfer contribute to development of antimicrobial resistance (Baxter et al., 2026). Overt or subtle signs of infection (section 2.6) may not be expressed fully because antigenic characteristics of bacterial cells are hidden by the biofilm EPS and instead, covert or subtle signs of infection (section 2.6) predominate, often waxing and waning

(Costerton et al., 1999, Wolcott et al., 2008). Clinical manifestations of this undulation are periods of increased exudate, maceration and tenderness followed by quiescent or stable phases, and because of antibiotic therapy failure, biofilm infections will demonstrate recurring symptoms (Wolcott et al., 2008, Costerton et al., 1999).

It is hypothesised that biofilm in a wound takes over the host immune response and locks the wound in a hyper-inflammatory state beyond the control of the host which serves to provide it with a consistent nutrient source derived from wound exudate (Wolcott et al., 2008). Interestingly, the inflammatory patterns found in chronic wounds are also to be seen in other chronic infections such as cystic fibrosis lung infections, periodontal disease and Crohn's disease/inflammatory bowel syndrome which are linked to biofilms, and they may also explain the common biochemical and cellular characteristics across different chronic wound aetiologies (Wolcott et al., 2008, Attinger and Wolcott, 2012).

Chronic wounds are complex entities and treatment protocols for their successful management require that the cause (for example, diabetes, venous insufficiency, external shear stress) be identified and treated, that patient/family concerns (for example, pain management, support systems, wellbeing etc.) be addressed and that local wound care (that is, debridement, inflammation/local infection, moisture balance and edge effect) be provided (Sibbald et al., 2021, Schultz et al., 2017, Orsted et al., 2017a). However, 'after controlling for these factors, biofilms are probably the most important single cause of persistent, delayed healing'(Schultz et al., 2017).

2.9 Treating biofilms in chronic wounds

In terms of treating biofilm in chronic wounds, biofilm-targeting regimens that include sharp debridement, long-duration systemic or topical antimicrobial therapy, cleansers and antimicrobial dressings are considered to be more effective than standard care (Metcalf et al., 2014, Cavallo et al., 2024, Razdan et al., 2022). Attinger and Wolcott proposed a strategy of regular debridement and appropriate topical biocide and systemic antibiotic therapy directed by polymerase chain reaction (PCR) techniques that identify the microbial species colonizing the wound (Attinger and Wolcott, 2012). The International Wound Infection Institute principles of best practice for wound infection in clinical practice 2022 update (Swanson et al., 2022) refer to BBWC, the goals of which are to 'physically remove the most tolerant microorganisms from the wound bed and create an environment that prevents or delays biofilm reformation' through multiple sessions of targeted wound hygiene, debridement strategies in conjunction

with therapeutic cleansing and use of topical antimicrobials/antimicrobial wound dressings. Where biofilm is suspected, cleansing should be therapeutic and vigorous, and the process may be facilitated by using agents such as surfactants (for example, Poloxamer 407, undecyclenamido-propyl betaine, macrogolum), super-oxidised solutions (for example, hypochlorous acid, sodium hypochlorite) and povidone iodine (Swanson et al., 2022). Antiseptic agents reported to have antibiofilm/biofilm inhibition properties include Alginogel, medical grade honey, Octenidine dihydro-chloride (OCT), PHMB and silver (for example, sulphadiazine, oxides, phosphate, sulphates and chlorides, elemental and nanocrystalline) (Swanson et al., 2022).

Indeed, BBWC is not a new modality and was the subject of a retrospective study of 190 patients with critical limb ischaemia (CLI) treated between 2002 and 2006 (Wolcott and Rhoads, 2008). The strategy incorporated sharp and ultrasonic debridement, and xylitol and lactoferrin as antibiofilm agents in conjunction with selective biocides including silver-impregnated dressings or cadexomer iodine. Antibiotics were considered as adjuncts and used in combination with the above elements. In addition, advanced technologies such as platelet-derived growth factor- β , cell therapy (for example, Dermagraft, Advanced Biohealing and Apligraf, Organogenesis) and hyperbaric oxygen (HBO) were incorporated if deemed necessary. Of the 190 patients included in the study, 146 (77%) showed complete healing. The study did not have a control group but when compared to a second retrospective study with the ‘closest match for patients included in this study’, the authors determined that BBWC was more effective than traditional wound care alone (Wolcott and Rhoads, 2008, Fife et al., 2002).

Other agents considered to have antibiofilm properties in that they destroy pre-formed biofilms include quorum sensing inhibitors (for example, azithromycin, bergamottin, usnic acid, quercetin, ellagic acid), EPS degrading enzymes (for example, dispersin B, deoxyribonuclease I), fatty acids (for example, caprylic acid, palmitic acid, myristoleic acid), metal chelators (for example, ethylenediaminetetraacetic acid [EDTA]), nitric oxide (for example, sodium nitroprusside, S-nitroso-L-glutathione, S-nitroso-N-acetyl penicillamine) and antimicrobial peptides (AMPs) (for example, Nisin A, human cathelicidin LL-37, human β -defensin 3, hepicidin 20) (Razdan et al., 2022).

In terms of these interventions though, there are concerns. Use of systemic antibiotics to treat certain wound aetiologies such as diabetic foot ulceration is not necessarily the preferred option because it is not supported by adequate evidence (Razdan et al., 2022). Systemic treatment of infection can be complex with risks of adverse effects, adverse drug interactions and inadequate drug concentrations reaching the local wound site, and the presence of biofilm in chronic

wounds can limit the effectiveness of antibiotic intervention (Razdan et al., 2022, Attinger and Wolcott, 2012). Topical delivery of antibiofilm agents either alone or in combination with antibiotics could potentially be beneficial in managing chronic wound infections, but some of the older topical antiseptics (for example, hydrogen peroxide, sodium hypochlorite and chlorhexidine) are non-selective and can be toxic to host cells involved in the repair process, and much of the evidence and support behind agents with antibiofilm properties appears to come from *in vitro* and animal studies, or from expert consensus/literature review, with few methodologically rigorous studies involving human participants (Razdan et al., 2022, Swanson et al., 2022, Wolcott and Rhoads, 2008, Weigelt et al., 2021). Furthermore, ‘there is no consensus on wound cleansing techniques (for example, passive soaking, swabbing, irrigation, or showering/washing), inconsistencies in procedural aseptic techniques (that is, sterile/surgical versus clean/standard), and antiseptic solutions abound in clinical practice’ (Swanson et al., 2022).

2.10 Identifying biofilm in chronic wounds

Determining the presence of biofilm infections can prove challenging and there are no indisputable diagnostic tests available at this point in time (Høiby et al., 2015, Wu et al., 2019a). The following subsections will briefly describe currently available detection methodologies.

2.10.1 Semi-quantitative cultures

Semi-quantitative cultures of wound samples obtained with the Levine or Z swab techniques are commonly used to facilitate estimation of bacterial load in chronic wounds (Serena et al., 2021, Bowers and Franco, 2020, Orsted et al., 2017a, Wu et al., 2019a). However, this methodology is only capable of identifying 1% of the bacteria present because firstly, biofilms contain bacterial cells existing in a stationary/slow growing or ‘viable but nonculturable (VBNC)’ state and cannot be grown on standard agar plates and secondly, in multispecies biofilms the culturability of one species can be affected by presence of others (Wu et al., 2019a). In addition, swabbing is non-invasive and can only collect surface-situated biofilm but not that located deeper in the tissues of the wound bed (Høiby et al., 2015, Johani et al., 2017, Percival et al., 2015b).

2.10.2 Metagenomics

Metagenomic methodologies using 16s ribosomal RNA or DNA can be used to identify pathogens in the biofilm phenotype (Wu et al., 2019a, Darvishi et al., 2021). They are more precise than standard culturing methods and can detect non-culturable cells and mixed species in biofilms (Darvishi et al., 2021). The DNA-based technologies do not distinguish between planktonic and biofilm phenotypes though, provide no cell viability data and are susceptible to environmental contamination, and some bacterial species cannot be detected with 16s RNA sequencing (Darvishi et al., 2021).

2.10.3 Microscopy

Another option for biofilm detection is to use microscopy (Oates et al., 2014, Høiby et al., 2015, Wu et al., 2019a). Confocal laser scanning microscopy and SEM are non-invasive techniques that are capable of detecting biofilms in tissue biopsy samples and are considered by ESCMID to be the most appropriate biofilm detection method (Wu et al., 2019a, Høiby et al., 2015). These techniques can allow visualisation of both biofilm EPS and aggregate structures, their morphology and size (Reichhardt and Parsek, 2019, Oates et al., 2014, Wu et al., 2019a). Confirmation of biofilm typically requires identification of at least two of the following criteria: microbial surface attachment, structured assemblies of bacterial cells and an exopolymer matrix (Oates et al., 2014).

2.10.4 Peptide nucleic acid fluorescence *in situ* hybridization

Peptide nucleic acid fluorescence *in situ* hybridization – a method of hybridising fluorescent labelled peptide nucleic acid (PNA) probes with bacterial ribosomal RNA has been used in conjunction with CLSM to investigate the spatial distribution and ecology of biofilms in chronic wound biopsies (Fazli et al., 2009, Kirketerp-Møller et al., 2008).

2.10.5 Emerging technologies

Other emerging techniques include wound blotting whereby nitrocellulose membranes are applied to wound surfaces and then stained for components of EPS, quantification of biofilm associated biomarkers (for example, extracellular DNA, quorum sensing molecules and MMPs), and transcriptomics which identifies the range of messenger RNA (mRNA) expressed

and allows identification of bacteria and description of their antibiotic resistance levels and biofilm formation tendencies (Darvishi et al., 2021, Wu et al., 2019a).

2.10.6 Clinical signs and symptoms of biofilm

Given the consensus that biofilm adversely affects wound healing trajectories (Schultz et al., 2017), a means of diagnosing or confirming their presence in chronic wounds should be a natural and important consideration. Yet with the exception of standard culturing, none of the methodologies described in subsections 2.10.1 to 2.10.5 are routine, and some are novel, still in relatively early stages of development. The most reliable and appropriate techniques would appear to be those that incorporate elements of advanced microscopy, but these are highly specialised and not available for routine diagnostic work in clinical microbiological laboratories, nor are they available for use at the bedside (Wu et al., 2019b, Høiby et al., 2015, Wei et al., 2019). Viable alternatives should therefore be sought.

One such alternative would be to look for signs and symptoms that point to biofilm in chronic wounds. Indeed, a consensus guideline from 2017 by Schultz et al. ranked the statement ‘In the absence of bedside diagnostic tests, specific signs and symptoms should be used to confirm biofilm presence’ as having strong agreement among a panel of 10 clinicians and researchers with expertise in laboratory and clinical aspects of biofilms (Schultz et al., 2017). Furthermore, the IWII principles for best practice for wound infection in clinical practice and guidelines for identification and treatment of biofilms in chronic wounds by Schultz et al. both provide lists of criteria potentially indicative of biofilm in a chronic wound (Swanson et al., 2022, Schultz et al., 2017). The IWII principles (Swanson et al., 2022) cite failure of appropriate antibiotic treatment, recalcitrance to appropriate antimicrobial treatment, recurrence of delayed healing on cessation of antibiotic treatment, delayed healing despite optimal wound management and health support, increased exudate/moisture, low-level chronic inflammation, low-level erythema, poor granulation/friable hypergranulation and secondary signs of infection while Schultz et al (Schultz et al., 2017) cite ineffective antibiotic and antimicrobial therapy, delayed wound healing, recurrent or worsening infections, profuse exudate, mild chronic inflammation and mild redness and swelling.

Visual cues are also promoted; in some cases, biofilms can present as a macroscopic and visible structure such as dental plaque, and there are those who describe a visible biofilm in wounds that takes the form of for example, translucent slimy patches, gel-like substances or cloudy, thin, slime material that is persistent, tolerant to antimicrobials and that will reform quickly

post debridement (Metcalf et al., 2014, Hurlow and Bowler, 2009, Metcalf and Bowler, 2016). However, it is also argued that biofilms in wounds cannot be observed because they mostly present as small aggregates less than 100µm in size and consisting of 100 cells or less, or as networks of patches over the surface of the wound that may not exceed a thickness of tens of micrometres (Lichtenberg et al., 2023, Percival et al., 2015a, Wei et al., 2019, Swanson et al., 2022, Schultz et al., 2017). In addition there is the possibility of mistaking slough, debris and exudate for biofilm, and while biofilm may cause the appearance of some wounds to change, observed changes may not necessarily be conclusive indicators of biofilm presence (Lichtenberg et al., 2023, Schultz et al., 2017, Swanson et al., 2022). Furthermore, slough presence and other visual markers of biofilm have been reported as overly subjective and without basis and objective criteria (Percival et al., 2012).

Diagnostic tools need supporting evidence that demonstrates their reliability and validity and promotes trust in end users.

Metcalf et al. proposed a clinical algorithm for identifying biofilm in chronic wounds in 2014 (Metcalf et al., 2014). The algorithm contains a mix of visual indicators (for example, a surface substance that detaches easily and atraumatically, but is persistent despite debridement and reforms quickly in the absence of frequent intervention), and indirect indicators (for example, poor response to antibiotics, poor response to dressings containing antiseptic agents [silver, iodine etc.], and favourable response to multimodal strategies [physical debridement, cleansing, topical antimicrobial agents and dressings]). However, this is a proposed algorithm, and it does not appear that any validation work has yet been carried out on it. Percival et al, have also suggested an identification algorithm (Percival et al., 2015b). This algorithm asks if the wound is failing to heal as expected, if all appropriate clinical diagnostic and therapeutic measures have been undertaken, if there is evidence of slough/necrotic tissue in the wound, if there are signs of local infection or inflammation, if the wound is responding to topical/systemic antimicrobial interventions and if the wound is showing evidence of granulation. The answer to each question is a dichotomous 'yes' or 'no' and depending on the answer, the user is either directed to continue with standard care protocols or to proceed to the next question in the sequence until they are directed to initiate a biofilm based management strategy. Again, it does not appear that any validation work has been carried out on this algorithm.

The last five sections have undertaken to look at the difference between acute wound and chronic wound infections and how infections manifest in chronic wounds. They have also

introduced biofilms, the concept of interest for this project and how they are hypothesised to adversely engage with the immune system to optimise conditions for their survival and contribute to delayed healing of these wounds in the process. Significantly, they have looked at treatment and identification strategies for biofilm in chronic wounds, illustrating some associated drawbacks.

In this next and penultimate section, accounting for the consensus that biofilm contributes to delayed healing in chronic wounds and the challenges around treatment and detection strategies as discussed in sections 2.9 and 2.10, the reader will be presented with the critical concerns that lead to the rationale for this project.

2.11 Thesis critical concerns and rationale

There is a paradigm that implicates biofilm in the delayed healing of chronic wounds, and the causative pathway is thought to be a takeover of the wound healing process by biofilm to create a persistent hyperinflammatory state which provides a consistent supply of nutrient material (Schultz et al., 2017, Metcalf et al., 2014, Attinger and Wolcott, 2012, Swanson et al., 2022, Goswami et al., 2023, Percival et al., 2012, Wolcott et al., 2008, Metcalf and Bowler, 2013).

Given that:

- biofilms are implicated in numerous other chronic bacterial infections,
- many signs and symptoms thought to be indicative of biofilm reflect both inflammatory dysfunction and the protective morphology of the biofilm
- and biofilm targeting wound management strategies are reported to have positive clinical outcomes (Wolcott et al., 2008, Attinger and Wolcott, 2012, James et al., 2008, Malone et al., 2017a, Goswami et al., 2023, Percival et al., 2012, Metcalf et al., 2014, Swanson et al., 2022),

this is not an unreasonable train of thought and arising out of this paradigm are many strategies and interventions for treating biofilm in chronic wounds.

However, in 2012, Attinger and Wolcott advised caution in terms of their approach to biofilm management (section 2.9), citing lack of randomised controlled trials but reporting ‘good research data and level 3 evidence’ (Attinger and Wolcott, 2012), and in 2017 Schultz et al. wrote that ‘data about incidence, susceptibility, and treatment of biofilms derived from experimental animal models and in vitro studies have helped shape general concepts about detecting and treating biofilm bacteria, but there are limits to the extent that this information can be extrapolated to clinical management. The clinical evidence base is poor, with limited

human studies and few controlled trials that provide scientifically acceptable data to inform clinical management' (Schultz et al., 2017). The rigorously researched IWII principles of best practice for wound infection in clinical practice 2022 update (Swanson et al., 2022) cites a 2008 retrospective case analysis (Wolcott and Rhoads, 2008) in terms of the BBWC concept (section 2.9), and as of the early 2020s, the evidence base for many antibiofilm agents and emerging novel therapies is still largely rooted in *in vitro* work or *in vivo* animal models (Razdan et al., 2022, Weigelt et al., 2021).

Furthermore, as outlined in section 2.10, routine quantitative culturing methodologies are limited in regard to biofilm detection, the optimal methodologies are expensive and not routinely available, novel diagnostic technologies are still in the early stages of development and clinical indicators thought to be indicative of biofilm presence in chronic wounds remain largely unvalidated.

Yet wound care journals host advertisements for products such as impregnated foam, silver, silver with surfactants and chelators, bioengineered honey and debridement/cleansing cloths, all of which are implied to have antibiofilm/biofilm management capabilities in chronic wounds (Keneric Healthcare, 2019, Convatec, 2016, Convatec, 2023, Convatec, 2019, H&R Healthcare, 2018, Medi, 2018, L&R Medical, 2018, Urgo Medical, 2022).

A critical concern then arises in terms of antibiofilm strategy effectiveness and good antimicrobial stewardship. If biofilm cannot be 'seen', that is, if its presence cannot readily be confirmed in chronic wounds, how can it be known if:

- it is actually there in the first place,
- it has been removed and that the employed antibiofilm strategies have been effective,
- and if removal of biofilm truly helps to draw the wound out of the inflammatory phase and facilitates the healing process to continue. Indeed, Kirketerp-Møller et al. point out in a perspective article from 2020 that despite having biofilm present, many chronic wounds still heal, and they question whether they interfere with the healing process at all (Kirketerp-Møller et al., 2020).

Consequently, by operating antibiofilm strategies at least partially in the dark is there a danger firstly, of administering sometimes unnecessary treatments to patients that are not completely without risk, and secondly, of compromising good antimicrobial stewardship principles.

A logical proposal that might help to address and possibly mitigate these concerns would be to investigate if a means of confirming biofilm in chronic wounds, in particular foot ulcers in persons with diabetes that is convenient and feasible to use at the bedside could be developed.

Clinical signs and symptoms are commonly incorporated into the diagnostic process and used to facilitate confirmation of a variety of conditions, for example wound infection (NERDS and STONEES), severity of community acquired pneumonia (CURB-65) and streptococcal pharyngitis (Centor criteria) (Woo and Sibbald, 2009, Patel, 2021, Wagner and Mathiason, 2008, Gardner et al., 2001). These sign and symptom-based tools do not require complex equipment or extensive training for their use and are thus inexpensive and easily available, indeed a significant requirement is that they have statistically proven ability to predict the condition or outcome of interest (Beattie and Nelson, 2006). As discussed in subsection 2.10.6, clinical signs and symptoms are frequently recommended and used to confirm presence of biofilm in chronic wounds, but extensive validation work has not yet been performed on them. It is therefore proposed that clinical signs and symptoms that have been subjected to a statistical validation process be used as the basis for a convenient, user-friendly means of predicting biofilm presence in chronic wounds.

2.12 Chapter conclusion.

In this literature review normal and abnormal wound healing processes, wound infections and biofilms have been explored, and biofilms have been characterised in terms of chronic wounds. A paradigm of thought has been outlined, positing that they likely affect healing trajectories of these wounds adversely and independent of other causative factors, and a hypothesis as to how they bring about this delay in healing and prolong chronicity has been presented.

The challenges associated with their identification have been illustrated as has the lack of validated, diagnostic tools in the clinical context. Furthermore, concerns have been expressed as to how the inability to practically and easily confirm their presence in a wound raises issues around antimicrobial stewardship, around unintended excessive use of interventions and strategies reported to have antibiofilm properties, and around effectiveness of these treatments, considering that much of the evidence in their favour comes from the findings of *in vitro* and *in vivo* animal studies translated to clinical scenarios.

It is proposed that the concerns that have been raised, these gaps in the knowledge around biofilm treatments and identification in chronic wounds, could be addressed by working toward developing a more user-friendly means of predicting biofilm in chronic wounds that can be used by clinical personnel at the bedside.

The aim of this PhD project is therefore to begin directing research toward efforts to develop an uncomplicated, inexpensive and user-friendly tool using clinical signs and symptoms

thought to be indicative of biofilm in chronic wounds, that can conveniently facilitate prediction of biofilm in DFU at the bedside.

The process for determining the statistical ability of a series of clinical signs and symptoms to predict an outcome of interest and developing them to the point where they can be widely used in practice is known as the Clinical Prediction Rule (CPR) approach and this will be described in the next chapter (chapter 3, section 3.2). Briefly, it is a complex, lengthy and resource intensive process and this original research project will for the first time consider it for use in the context of predicting biofilm presence in chronic wounds.

It is very important that any research endeavour be carefully planned and designed in advance. According to Dublin City University Library, flawed or underdeveloped methodology will diminish the findings of a study and its overall value (Dublin City University (DCU) Library, 2023). In an article on research misconduct posted on The Embassy of Good Science platform, Lechner et al. wrote that the scientific method and the integrity of the research record is dependent on proper conduct of research and significantly, they point out that behaviours such as conscious minor breaches of research integrity and poor methodologies are more frequent than explicit research misconduct practices and are possibly more harmful to science (Lechner et al., 2021). Furthermore, Minogue et al. write that 85% of health research expenditure is potentially wasted because of, among other factors such as failure to publish findings and poor reporting of published findings, poor study design and conduct (Minogue et al., 2018).

Any predictive tool intended for use in clinical practice must be grounded in robust science and to immediately engage with the CPR process in this (or any) context without first investing significant time and effort in developing a feasible methodological protocol has the potential to be disastrous from methodological rigour/value of findings and research waste perspectives. It is important to emphasize at this point then that the focus of this PhD project will be on laying groundwork and developing a workable methodological protocol that can ensure smooth, efficient and resource-friendly running of the process in real life rather than on actually attempting to create a predictive tool that can indicate presence of biofilm in chronic wounds.

3 Chapter Three: Thesis methods and results

3.1 Introduction

In the previous chapter the concept of chronic wounds, in particular diabetic foot ulceration and those of chronic wound infections and bacterial biofilms were introduced. Gaps in the knowledge in terms of a practical means to indicate biofilm presence in chronic wounds at the bedside were revealed and consequent critical concerns were highlighted, serving as a rationale to advocate for the proposed solution of a predictive tool for biofilm in chronic wounds based on clinical signs and symptoms. This chapter will outline the methodological process to develop a protocol that could be used to create a prototype of this tool. Firstly, an introduction to the clinical prediction rule approach that guides this project's methodology will be presented. This will be followed by descriptions of the phases that comprise the methodology itself and their respective findings (Table 1):

- Phase 1: A scoping review of the literature to identify all possible clinical signs and symptoms reported to be indicative of biofilm in chronic wounds.
- Phase 2: An electronic Delphi exercise in which an international panel of wound care clinicians are asked to refine the findings of the scoping review by selecting the signs and symptoms they think are most likely to be potential indicators of biofilm in these wounds.
- Phase 3: A protocol development phase in which the processes for a clinical study to determine the statistical abilities of clinical signs and symptoms reported in the literature to indicate presence of biofilm in chronic wounds is created.
- Phase 4: An investigative site access phase wherein the regulatory, ethical and data protection systems that lead to acquisition of the necessary approvals for a clinical study will be researched to find a timely and efficient pathway through.
- Phase 5: A laboratory phase in which a procedure for highlighting biofilm morphological features using a series of fluorescent stains will be researched.

The reader should understand that these phases operate interdependently, and it would be better that they were not viewed as separate entities.

The scoping review of phase 1 forms the foundation for the methodological process as a whole and its findings feed the electronic Delphi process of phase 2. In turn, the findings of the electronic Delphi process feed the protocol development work of phase 3 which must pass through the regulatory systems explored in phase 4.

Therefore, with the aim of guiding the reader through the methodological processes for this project in a more efficient and intuitive manner, the results/outputs for a phase will be presented directly after the methods reported for that phase rather than having separate methods and results sections.

Table 1. Applying the derivation phase of the Clinical Prediction Rule approach to this PhD thesis.

Clinical Prediction Rule Approach				
Phase 1	Phase 2	Phase 3, 4 & 5		
Scoping review	Electronic Delphi	Clinical Study		
Protocol/Methods	Protocol/Methods	Methods		
Results	Results	Clinical Protocol. Wound assessments and tissue biopsy for statistical validation of clinical signs and symptoms.	Regulatory affairs/ Site access pathway.	Biofilm visualisation: alternative fluorescent staining procedure to PNA FISH for identifying biofilm morphological characteristics.

PNA/FISH = Peptide nucleic acid/fluorescence *in situ* hybridisation

3.2 Clinical prediction rules

Traditionally, the art of clinical decision-making throughout the different fields of medicine relied on intuition, the clinician's personal experience and expert opinion to determine the most appropriate evaluative procedures to be used, their interpretation, and which interventions were subsequently needed (Hajjaj et al., 2010, Beattie and Nelson, 2006). In 1996, Sackett et al. described evidence-based medicine (EBM) as 'the conscientious, explicit and judicious use of current best evidence in making decisions about the care of individual patients,' and said that

individual clinical expertise needed to be combined with systematically obtained, best available external clinical evidence (Sackett et al., 1996, Kirsi et al., 2023). Today, clinical prediction rules have become a useful means of integrating research into clinical decision making, helping to speed up the process and reduce risk of underlying bias (Beattie and Nelson, 2006, Cook, 2008). Clinical prediction rules have been defined as ‘a process by which combinations of clinical findings that have been statistically demonstrated to be meaningful predictors of a condition or outcome of interest are used to categorise a heterogeneous group of patients into subgroups based on a shared likelihood of the presence of that condition or outcome’ (Beattie and Nelson, 2006). More simply, they aim to estimate the probability of a clinical condition or outcome by considering a small number of valid indicators or predictive factors, typically three or more which could be patient characteristics, clinical signs or investigative results, serving to mitigate the uncertainty intrinsic to medical practice (Cowley et al., 2019, Wasson et al., 1985, Adams and Leveson, 2012, Cook, 2008). These tools are designed to serve in capacities of:

- diagnosis where they comprise predictive factors intended to facilitate diagnosis of or screening for a specific condition (for example, the Well’s rule for diagnosis of deep venous thrombosis [DVT])
- prognosis where they comprise predictive factors relating to measuring changes in patient status with time (for example, the European system for cardiac operative risk evaluation II (EuroSCORE II) for long-term cardiovascular prognosis)
- prescription where they comprise factors that can predict most effective and appropriate interventions (for example, determining which patients with back pain would benefit most from spinal manipulation and stabilisation exercises) (Cook, 2008, Beattie and Nelson, 2006, van Smeden et al., 2021, Haskins and Cook, 2016).

The development process for CPRs starts with a definition of an outcome of interest, for example the presence or absence of a condition or disease and proceeds through a series of defined methodological stages of derivation, validation and impact analysis (Adams and Leveson, 2012, Cook, 2008, Beattie and Nelson, 2006, Cowley et al., 2019).

3.2.1 Derivation

The foundational stage or derivation stage, also referred to as creation of the rule, requires that variables likely present in the population under investigation and having predictive value for the outcome of interest be determined (Beattie and Nelson, 2006, Childs and Cleland, 2006). These variables may originate from sources such as clinical findings, history or investigation

results, literature review, expert opinion or focus groups of patients, but critically, their predictive properties must be *statistically determined* (Beattie and Nelson, 2006, Cowley et al., 2019). Ideally, a cross-sectional experimental design that will help to determine the nature of the statistical relationship (if any) between the predictor variables and the outcome of interest best serves this stage in terms of a diagnostic test (Cowley et al., 2019, Beattie and Nelson, 2006). Within such a design, a sample of patients likely to have or develop the outcome of interest are assessed at baseline to determine whether or not they exhibit each of the potential predictors (Childs and Cleland, 2006). At this point, assessors must be blind to knowing the status of the patient in terms of the outcome of interest which is established against an accepted reference criterion or gold standard (for example, biopsy or imaging data) by a second assessor who is blind to the outcome of the baseline assessment (Beattie and Nelson, 2006, Childs and Cleland, 2006). The findings of the baseline assessment or index test and those of the gold standard or reference standard test are presented on a 2 X 2 table (subsection 3.5.2) and the degree of agreement between the index and reference tests will be a reflection of diagnostic accuracy of the predictors (Florkowski, 2008).

There are some concepts that are fundamental in regard to establishing a statistical relationship between a predictor variable and an outcome of interest. The remainder of this section will be devoted to providing basic explanations of these concepts so that the reader will gain some understanding of what is required to establish a true and solid mathematical foundation for a CPR.

Validity is the degree to which a test measures what it is expected to measure, that is, validity refers to the accuracy of a test and is measured in terms of sensitivity and specificity (Parikh et al., 2008).

Sensitivity is a measure of how well a test can identify people who have the outcome of interest while specificity pertains to how well a test can identify people who do not have the outcome of interest (Loong, 2003). A false positive or type I error occurs when a test determines that a patient has the outcome of interest when in actuality they don't, and a false negative or type II error occurs when a test determines that a patient does not have the outcome of interest when in actuality they do (Loong, 2003).

Positive predictive value (PPV) is a measure of probability that a positive test result will be correct, that is, it looks at all positive values (both true and false positives), and negative predictive value (NPV) is a measure of probability that a negative result will be correct, that is, it looks at all negative values (both true and false negatives) (Table 2) (Loong, 2003).

Table 2. Overview of the statistical characteristics of a diagnostic test.

Concept	Focus	Explanation	Formula
Sensitivity	Truly sick	How well a test correctly identifies people who are truly sick. The test will correctly identify a proportion of the truly sick (TP). But it will also but will also fail to correctly identify a proportion of the truly sick (FN).	$TP/TP + FN$
Specificity	Truly well	How well a test correctly identifies people who are truly well. The test will correctly identify a proportion of the truly well (TN). But it will also fail to correctly identify a proportion of the truly well (FP).	$TN/TN + FP$
False negative rate (Miss rate) 1 - Sensitivity	Truly sick	Probability of a negative test in persons who are truly sick. Or probability that a true positive will be missed by the test.	$FN/FN + TP$
False positive rate 1 - Specificity	Truly well	Probability of a positive test in persons who are truly well	$FP/FP + FN$
Positive predictive value	<u>All</u> positive results	Probability that the positive result of a test is correct.	$TP/TP + FP$
Negative predictive value	<u>All</u> negative results	Probability that the negative result of a test is correct.	$TN/TN + FN$

TP = True positive, FN = False negative, TN = True negative, FB = False positive

A risk factor (often identified by examining relative risk or odds ratio), can be defined as an element that increases the risk or susceptibility for an outcome of interest for example, weight stigma is a risk factor in the development of eating disorders, and they are often reported to be reliable predictors of clinical outcomes (Merriam-Webster, 2024, Grobman and Stamilio,

2006). However, even though a risk factor may have a strong association with an outcome of interest, it may not be a good predictor if its prevalence and the prevalence of the outcome are low (Grobman and Stamilio, 2006). Thus a good predictor in addition to having a strong association with an outcome, must be able to discriminate accurately between patients with and without the outcome of interest in terms of positive predictive value (Grobman and Stamilio, 2006).

High sensitivity of a predictor is the ideal property of a ‘rule out’ test while high specificity is the ideal property of a ‘rule in’ test and there is usually a trade-off between the two; as the sensitivity increases the specificity decreases and vice versa (Florkowski, 2008, Grobman and Stamilio, 2006, Chubak et al., 2012).

Sensitivity and specificity data about a predictor can be combined and likelihood ratios calculated which describe how that predictor will raise or lower the likelihood that the outcome of interest is present (Beattie and Nelson, 2006, Childs and Cleland, 2006). Ratios can be positive or negative with those greater than 1.0 increasing likelihood and those less than 1.0 reducing likelihood (Childs and Cleland, 2006). Accuracy of a predictor is considered substantial if a positive likelihood ratio exceeds 10 or a negative likelihood ratio is less than 0.10, moderate if a positive likelihood ratio falls between 5–10 or a negative likelihood ratio falls between 0.2–0.1, and low if a positive likelihood ratio falls between 2–5 and a negative likelihood ratio falls between 0.5–0.2 (Childs and Cleland, 2006, Beattie and Nelson, 2006).

Receiver-operating characteristic (ROC) curves illustrate this trade-off and are constructed by plotting sensitivity or true positive data on the y-axis versus 1-specificity or false positive data across a wide range of values (Grobman and Stamilio, 2006, Florkowski, 2008). Briefly, highly accurate predictors generate points close to the upper left corner of the plot while those with low accuracy (that is, having discriminative no greater than chance) will generate points close to the 45-degree diagonal, but the optimal discriminatory point can be arbitrary and influenced by severity of outcome and risk of test (Grobman and Stamilio, 2006). Area under the curve can also be used to express accuracy (Florkowski, 2008).

Statistical associations between predictor variables and outcomes of interest can also be made through univariate or multivariate statistical tests both of which have advantages and disadvantages and are described by Grobman and Stamilio in the remainder of this section (Grobman and Stamilio, 2006). For the former, strength of association can be determined with tests such as the Student’s *t* test and chi-square analysis and those predictors with significant associations can be used in the final model. The outcome of interest can be predicted by a score based on the number of predictors observed (for example, 0 = no predictors, 1 = one predictor

etc.). Predictors may provide different contributions to a final score and be weighted accordingly (for example, predictor a = 1 point, predictor b = 2 points etc.) or they may be categorised depending on the extent of their presence or severity (for example, predictor a may have a range of scores from 0 – 5). The advantages to models based on univariate analysis include ease of translation to the bedside and clinicians only need to know the predictors and the scoring system. Final scores can be obtained quickly and easily and have a limited range of values and the model itself typically has a limited range of outcomes. However, this method may include factors that are related to one another and are not independently associated with the outcome thus reducing predictive accuracy and additionally, points awarded to each factor in a model can be done so arbitrarily (for example, similar points can be awarded to factors with different predictive abilities).

Multivariate analysis on the other hand helps to minimize the limitations of univariate methodology, in particular those in regard to assessing the association of each independent variable with the outcome of interest. This means that prediction tools can be more strongly based both on relevance of included factors and the magnitude of their contributions. Logistic regression suits dichotomous outcomes and associations are expressed as odds ratios which are widely used in medicine and are easy to interpret. Stepwise logistic regression uses predetermined probabilistic criteria to determine which factors will be used in a final model. However, they may not take clinical experience into account, and they are not always considered clinically useful. In some models, regression coefficients are provided, and users construct probabilities for their own patients while in others, points are assigned to each predictive factor and coefficient calculations need not be carried out. In terms of disadvantages, it can be laborious if not impossible to search for interactions between predictor variables if those variables are numerous. Additionally, logistic regression makes assumptions about the linearity of relationships between predictor variables and outcomes of interest that may not always exist in reality. It is possible to investigate for these relationships and attempt to resolve them but again, the procedures are time consuming and may not always be successful.

Alternative methodologies that can account for these non-linear relationships include neural networks, predictive nomograms and classification and regression tree (CART) analysis.

Statistical analysis at this stage of the process do not provide evidence for clinical use, rather it allows investigators to assess whether predictors have a reasonable degree of diagnostic accuracy (Beattie and Nelson, 2006).

3.2.2 *Validation & Impact analysis.*

Clinical prediction rules are well known to perform better in the original dataset from which they were derived, and so the purpose of the validation phase in clinical prediction rule development is to demonstrate evidence of reproducible accuracy in ‘plausibly related or ‘similar but different’ individuals’ (Cowley et al., 2019, Beattie and Nelson, 2006). The validation phase can be divided into four levels as proposed by McGinn et al. and reported by Beattie and Nelson (Beattie and Nelson, 2006, McGinn et al., 2000). Level four validation is the most basic and serves to provide preliminary information in terms of the new clinical prediction rule’s stability in a limited and well defined population and under very restricted conditions. Split half of the original dataset or retrospective data can be used at this stage. Level three validation requires a new sample with the aim of determining stability of the model in a ‘different but similar’ sample scenario. Studies should be prospective and involve the same examiners. Strong level three validation provides cautionary support for use of the rule in other, similar samples of patients. Level two validation is an attempt to see if the new rule will prove stable and provide similar results across a variety of patients and clinicians. Accuracy should be consistent in either one prospective study including a variety of patients and clinicians or in a series of smaller studies with variable characteristics in included participants. If the evidence at this level is strongly supportive of the tool, it may be considered for use in different settings with similar patients and clinicians to those assessed.

Finally, level one validation assesses whether a clinical prediction rule improves practice in general, if it is bringing about changes in clinical behaviour and crucially, if it is improving patient outcomes. At this stage, the rule should have level two evidence and at least one impact study that illustrates change in clinician behaviour and improved cost-effectiveness and streamlining of patient care in terms of better outcomes, lower costs and fewer adverse events. This level of validation supports use of the rule in a wide variety of settings knowing that their incorporation into practice will be beneficial.

3.2.3 *Real world example: The Ottawa Ankle rules.*

The Ottawa ankle rule is an example of a clinical prediction rule used for diagnostic purposes and its origins lie in the identification of a significant clinical problem in Canada and the USA (Stiell, 1996). Physicians were ordering foot and ankle x-rays frequently for common ankle injuries which by themselves were inexpensive, but cumulatively were adding significantly to rising health care costs, and in his 1996 report, Stiell (Stiell, 1996) estimated that \$500,000,000

was being spent annually on ankle radiography across the two countries. A group of Ottawa emergency physicians and researchers subsequently began work on a multiphase project that spanned four years. Initially in phase zero, they identified that over 95% of ankle injury patients seen at three Ottawa emergency departments underwent ankle and/or foot radiography but only 15% of the radiographs demonstrated an abnormality. In addition, they noted that physicians were not confident in their clinical ability to distinguish between fractures and non fractures. In phase 1 of the project, they evaluated 750 emergency department patients for 32 clinical findings, and of these patients a sample of 100 was selected and independently examined by two physicians to select the most reliable of the findings. They ultimately developed two rules for ruling in a) ankle x-rays and b) foot x-rays based on multivariate analyses and clinical experience. In phase two the group validated the rules prospectively and refined them in another sample of 1,485 patients with the finding that the rules were 100% sensitive for selecting fractures of the malleoli and midfoot. Phase three involved putting the rules into practice and they were taught to clinical personnel in the Ottawa Civic Hospital emergency department over a period of five months. The group found that compared to baseline and a community hospital control, radiography in a sample of 593 patients was reduced by 28% and 14% for ankle and foot radiographs respectively, and the rules identified all malleolar and midfoot fractures ($n = 93$) accurately. Compared to patients who did undergo radiography, non-radiography patients spent 36 minutes less in the emergency department, had lower radiography and doctor's visit costs, were no more likely to have subsequent x-ray tests and were equally satisfied with care received. The group also completed a multi-centre study in eight diverse Ontario hospital settings that included 12,000 patients with ankle injuries. The study found large reductions in x-ray examinations for all settings (small and large community and teaching) and all types of physician included (full-time, emergency, house staff and family) with no increase in missed fractures.

The group caution that the tool should not be used to completely override clinical judgement and indeed, despite the large degree of validation work it is not without criticism (Stiell, 1996, Beattie and Nelson, 2006). Brehaut et al. carried out a postal survey of 399 randomly selected emergency physicians and wrote that despite its wide use by most physicians, it is not considered to be a major determinant in decision making implying that its impact may actually be negligible (Beattie and Nelson, 2006, Brehaut et al., 2005). However, a systematic review and meta-analysis from 2022 investigating the diagnostic accuracy of the tool (Gomes et al., 2022) found it to have high sensitivity, making it capable of ruling out ankle fractures with confidence, but lower specificity which increases the likelihood of false-positive outcomes.

The authors concluded that their data supports use of the Ottawa Ankle Rules as a ‘cost effective tool to reduce unnecessary radiographic referral, when used in conjunction with the emergency clinician’s clinical reasoning and judgement. Implementation and uptake of the tool will improve efficiency, lower medical costs and reduce waiting times for those attending health services following ankle trauma’ (Gomes et al., 2022).

Given the complexity of the clinical prediction rule approach with its different phases and multiple levels of validation, and taking the example outlined above into consideration, it is apparent that its application requires ongoing commitment and is resource intensive in terms of time, personnel, finances and sample sizes. Indeed, sample sizes for the derivation phase alone have been reported to range from 54 to almost 600 patients, and in some cases thousands of consenting participants may be required (Beattie and Nelson, 2006, Childs and Cleland, 2006, Hartling et al., 2002, Hicks et al., 2005, Wainner et al., 2005, Tseng et al., 2006, Kuijpers et al., 2006).

The implications of a CPR for biofilm in chronic wounds could be enormous in terms of personal, social and economic impact, and inappropriate use of antimicrobial therapy strategies could be significantly mitigated. Therefore, it is imperative that the methodology to develop such a tool be carefully designed and have a solid foundation that will hold up well to future critical appraisal and this thesis will be instrumental in establishing this foundation.

In light of these resource commitment and implications factors the reader is again reminded that this PhD project is primarily a protocol development exercise. This research work is not intended to, nor does it have the scope to incorporate the CPR approach to its fullest extent, but what it will do is take guidance from the derivation or creation of the rule phase to develop this foundational methodology.

The next sections will document the five methodological phases of this PhD project.

3.3 Thesis phase 1: A scoping review of the literature

3.3.1 Introduction

Evidence synthesis has been defined by the Cochrane Collaboration as the process of ‘combining information from multiple studies investigating the same topic to comprehensively understand their findings’ (Cochrane Collaboration, 2023). The Royal Society defines it as ‘the process of bringing together information from a range of sources and disciplines to inform debates and decisions on specific issues’ (The Royal Society).

The well-known systematic review process embodies the concept of evidence synthesis and seeks to answer specific and focused research questions by gathering evidence that meets pre-specified eligibility criteria, minimising bias by using ‘explicit, systematic methods documented in advance with a protocol’ (Cumpston et al., 2024). The methodology requires that investigators develop a research question *a priori*, and based on that research question, a systematic search of the literature must be created and conducted. The search findings must then be screened against study eligibility criteria using pre-specified selection and assessment methods and finally, the procedures for extracting data from eligible material and the approach to its analysis must be detailed (Yuan and Hunt, 2009, Tawfik et al., 2019). Furthermore, a key aim within the systematic review process is to conduct meta-analysis – ‘the statistical combination of results from two or more separate studies’ to improve precision, answer questions that individual studies do not pose and to resolve conflicting claims (Deeks et al., 2024). The appropriateness of incorporating meta-analysis into a systematic review is contingent on factors such as clinical heterogeneity, statistical heterogeneity and risk of bias within the included studies (Lensen, 2023).

However, systematic reviews are designed to use data from a specific range of quality-assessed study designs (ideally, randomised controlled trials) to answer narrow clinical research questions that contain clearly defined elements of population, intervention, comparator and outcome and this approach may not be appropriate for answering questions around broader issues such as clarification of concepts, themes or definitions or literature mapping exercises (Russell et al., 2009, Smith and Duncan, 2022, Arksey and O'Malley, 2005). In particular, Munn et al. (Munn et al., 2018) outlined the following indications for adopting a systematic review methodology:

- To uncover the international evidence

- To confirm current practice/address any variation/identify new practices
- To identify and inform areas for future research
- To identify and investigate conflicting results
- To produce statements to guide decision making.

In 2005, Arksey and O'Malley described scoping studies as exercises that address broad topics, often including many study design types but without any quality appraisal of those included studies and suggested that they might be undertaken for purposes such as mapping research activity in a field of study, determining the value of undertaking a full systematic review, summarising and disseminating research findings or identifying research gaps in the literature (Arksey and O'Malley, 2005). In addition they outlined a methodological framework that included stages to identify a research question, identify relevant studies, select studies, chart data, collate, summarise and report results and to consult with relevant stakeholders (Arksey and O'Malley, 2005). Levac et al. subsequently proposed recommendations to enhance the framework at each stage that included clarifying/linking the study research question and purpose, balancing the breadth and comprehensiveness of the scoping process with feasibility, using an iterative team approach to study selection and data extraction, incorporating numerical summaries and qualitative thematic analysis in results reporting, considering the implications of study findings to policy, practice, or research and incorporating consultation with stakeholders as a required knowledge translation component (Levac et al., 2010).

Since 2015, the Joanna Briggs Institute (JBI) has been working to refine the methodology of this type of evidence synthesis further, has produced corresponding guidance emphasising rigour of conduct, transparency and trustworthiness and refined the nomenclature from 'systematic scoping review' to simply 'scoping review' (Peters et al., 2024 , Peters et al., 2021). In addition the Preferred Reporting Items for Systematic Reviews (PRISMA) statement has been extended to scoping reviews – the PRISMA ScR, facilitating researchers in maintaining consistency with JBI scoping review methodology (Peters et al., 2024 , Peters et al., 2021).

Scoping reviews have been formally defined as 'a type of evidence synthesis that aim to systematically identify and map the breadth of evidence available on a particular topic, field, concept or issue, often irrespective of source (that is, primary research, reviews, non-empirical evidence) within or across particular contexts (Munn et al., 2022). Scoping reviews can clarify key concepts/definitions in the literature and identify key characteristics or factors related to a concept, including those related to methodological research' (Munn et al., 2022).

Currently, their indications are to (Munn et al., 2018):

- Identify the types of available evidence in a given field
- Clarify key concepts/definitions in the literature
- Examine how research is conducted on a certain topic or field
- Identify key characteristics or factors related to a concept
- Act as a precursor to a systematic review
- Identify and analyse knowledge gaps.

It has been suggested that when deciding between a systematic or scoping review approach to evidence synthesis, a researcher should consider the indications as described above for each approach, taking into account the question they wish to have answered and the purpose of their review (Munn et al., 2018). Most importantly, the researcher should ask if the findings of their review are intended to either answer a clinically meaningful question/provide evidence to inform practice, in which a systematic review approach should be taken, or if they are intended to identify, map, report and/or discuss characteristics, thus necessitating a scoping review approach (Munn et al., 2018).

This PhD project is directed toward answering a clinically meaningful question which has the potential to inform practice. However, this first phase is only concerned with collating a comprehensive list of clinical signs and symptoms that have been reported in the literature to be indicative of biofilm in chronic wounds. In terms of indications for choosing an evidence synthesis approach (Munn et al., 2018), the research question does not fit any of those outlined for a systematic review. However, upon looking at indications for a scoping review it could be interpreted as an effort ‘to identify key characteristics or factors related to a concept’. In addition, preliminary searches of the literature revealed that much of the data required to answer the research question for this phase tended to come from material such as case series, editorials and review articles, typically located toward the base of the evidence pyramid, meaning that a systematic review approach with restrictive eligibility criteria in terms of included study design might lead to relevant articles being excluded (Murad et al., 2016).

For these reasons, it was decided to adopt a scoping review approach rather than a systematic review approach to answer the research question for phase 1 and collate a list of signs and symptoms from the literature reported to be indicative of biofilm in chronic wounds.

3.3.2 Scoping review published protocol

The following is a protocol for this phase of the project and it has been published on the Health Research Board's (HRB) open access platform (Ivory et al., 2021).

Database search strategies and a completed Peer Reviewed Electronic Search Strategy (PRESS) form can be found in Appendix 1.

HRB Open Research

A scoping review protocol to identify clinical signs, symptoms and biomarkers indicative of biofilm presence in chronic wounds

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Roles: Conceptualization, Funding Acquisition, Investigation, Methodology, Project Administration, Supervision, Validation, Visualization, Writing – Original Draft Preparation, Writing – Review & Editing

Abstract

Introduction: Wound healing is characterised by haemostatic, inflammatory, proliferative and remodelling phases. In the presence of comorbidities such as diabetes, healing can stall and chronic wounds may result. Infection is detrimental to these wounds and associated with poor outcomes. Wounds are contaminated with microbes and debris, and factors such as host resistance, bacterial virulence, species synergy and bioburden determine whether a wound will deteriorate to critically colonised/infected states. Biofilms are sessile microbial communities, exhibiting high-level antibiotic tolerance and resistance to host defences. Biofilm in critically colonised wounds can contribute to delayed healing. Little is known about clinical presentation and diagnosis of wound biofilms.

Objective: To identify from the literature clinical signs, symptoms and biomarkers that may indicate biofilm in chronic wounds.

Methods: This review will be guided by the Preferred Reporting Items for Systematic Reviews extension for Scoping Reviews (PRISMA-ScR), and the Joanna Briggs Institute Manual for Evidence Synthesis.

Studies of any design in any language recruiting adult patients with venous, diabetic, pressure or mixed arterial-venous ulcers and reporting data on clinical signs/symptoms of biofilm are eligible.

Searches of Medline, Embase, CINAHL, Cochrane Central and BASE will be conducted from inception to present. Reference scanning and contact with content experts will be employed.

Title/abstract screening and full text selection will be executed by two reviewers independently. Discrepancies will be resolved by discussion between reviewers or through third party intervention. Data will be extracted by a single reviewer and verified by a second.

Clinical signs and symptoms data will be presented in terms of study design, setting and participant demographic data.

Discussion: Understanding biofilm impact on chronic wounds is inconsistent and based largely on *in vitro* research. This work will consolidate clinical signs, symptoms and biomarkers of biofilm in chronic wounds reported in the literature.

Keywords: Chronic wound, Wound healing, Infection, Biofilm, Clinical signs and symptoms

Introduction

Wound healing occurs via a complex sequence of events which, under normal circumstances proceed in an orderly fashion through haemostatic, inflammatory, proliferative and remodelling/maturation phases to restore cutaneous integrity and barrier function. However, in the presence of complicating factors such as diabetes or chronic venous insufficiency, the healing process can break down and the wound becomes chronic, failing to heal in a timely manner.^[1, 2] There is a lack of consensus regarding the definition of chronic wounds and they have for example, been described as ‘wounds that have not proceeded through an orderly and timely reparation to produce anatomic and functional integrity after 3 months’, ‘wounds that lack a 20-40% reduction in size after 2-4 weeks of optimal treatment or when there is not complete healing after 6 weeks’, or simply as ‘wounds that fail to proceed through the normal phases of wound healing in an orderly and timely manner’^[2, 3]. Typically, these wounds include but are not limited to venous, diabetic and pressure aetiologies^[4]. Chronic wounds of mixed aetiologies were estimated to have a pooled prevalence of 2.21 per 1000 population in a 2019 meta-analysis of three studies, while a second meta-analysis from the same year including nine studies estimated the pooled prevalence of chronic leg ulcers to be 1.5 per 1000 population^[5]. Chronic wounds are burdensome to the individual in terms of finances and quality of life, and to healthcare systems. Unhealed diabetic foot ulcers (DFU), venous leg ulcers (VLU) and pressure ulcers (PU) cost the United Kingdom National Health Service (NHS) approximately £4 billion in 2017-2018, or an average of £6305 per patient.^[6, 7]

Infection commonly affects chronic wounds and is associated with poor clinical outcomes^[8]. The risk of hospitalisation for patients with a DFU is increased by a factor of 50 while the risk of lower-extremity amputation is 150 times higher if their wounds become infected.

Development of infection in chronic wounds is a complex process. Multiple factors including virulence of colonising organisms, synergy between multiple microbial species, bioburden and host resistance interact with each other and determine whether a wound will progress from a non-threatening, contaminated/colonized state through to critically colonized or infected states. [9]

Bacteria can manifest in wounds as a free-floating planktonic phenotype or as a sessile biofilm phenotype [10]. Biofilms occur when microbial cells organise themselves into aggregates or communities encased in a self-produced polymeric matrix which typically attach to surfaces. Biofilms exhibit high levels of tolerance to antimicrobial agents and host defences [11]. When they form in critically colonized chronic wounds, healing stalls and the wound remains stuck in the inflammatory phase [12-16].

In 2017, a panel of specialists, chosen for their expertise in chronic wounds and biofilms, for their scholarly activity and publication record, issued a consensus document [17]. The document aimed to clarify the role of biofilms in clinical practice, help clinicians to recognize biofilms in chronic non healing wounds and optimise patient management. A modified Delphi process was used to achieve consensus on a series of statements formulated to address issues in ten areas relevant to management of non-healing chronic wounds. Five-point Likert scales of agreement (1 = disagree strongly – 5 = agree strongly) and ranking (1 = not important – 5 = most important) were used to score the statements. There was strong agreement (mean 4.0, standard deviation [SD] 0.82) that specific clinical signs and symptoms should be used to confirm presence of biofilm in the absence of diagnostic bedside tests. Clinical features, such as a recurring gelatinous material on the wound edge, have been proposed as surrogate markers of wound biofilm but there was weak agreement (mean 3.6, SD 1.5) that the clinical signs and symptoms that could indicate the presence of biofilm. Little is known about presentation and diagnosis of wound biofilms and knowledge of their characteristics is limited [18]. Generally, biofilms are difficult to diagnose and currently no guidelines exist to help clinicians and microbiologists in diagnosis and treatment [19].

In addition, little quantitative work has been done with respect to clinical signs and symptoms of biofilm in chronic wounds, especially in human patients, and existing published research is mainly observational rather than incorporating more rigorous study designs [12, 17, 18, 20, 21].

For these reasons we suspect that a rigorous systematic review with a focused research question and strict criteria with respect to eligible study design may be too exclusive and fail to answer the research question.

A scoping review methodology will therefore be employed to identify any associated clinical signs and symptoms thought to determine the presence of biofilm in chronic wounds.

The research question for the study is: what clinical signs, symptoms and biomarkers are proposed within the literature to determine the presence of biofilm in chronic wounds?

Methods

The Preferred Reporting Items for Systematic Review and Meta-Analyses extension for Scoping Reviews (PRISMA-ScR) statement, the Joanna Briggs Institute Manual for Evidence Synthesis will guide this work ^[22, 23].

Inclusion criteria

Eligible participants will be adult (18 years +). Eligible wound aetiologies will be venous leg ulcers (VLU), diabetic foot ulcers (DFU), pressure ulcers (PU) and/or mixed arterial/venous leg ulcers (MAVLU), treated in any setting.

Databases will be searched from inception to present without limits on language. Study designs including but not limited to systematic reviews, randomized controlled trials (RCTs), controlled clinical trials, cohort studies, case series, case reports, letters to the editor with relevant data and editorials will be included. These articles must report any clinical signs, symptoms and/or biomarkers, validated or otherwise, thought to be associated with the presence of biofilm in chronic wounds.

Exclusion criteria

Patients with wounds resulting from burns, malignant fungating wounds, wounds secondary to conditions such as rheumatoid arthritis or pyoderma gangrenosum are ineligible for this study.

Search strategy

A search strategy will be developed in Medline, peer reviewed according to Peer Review of Electronic Search Strategies (PRESS) guidelines ^[24] and adapted for use in Embase, CINAHL, Cochrane Central, and The Bielefeld Academic Search Engine (BASE).

Reference scanning

Reference lists of included articles will be scanned to locate subsequent, potentially relevant articles.

Content experts and organisations

Content experts and relevant organisations will be consulted to obtain information about unpublished or ongoing studies and where applicable, to request access to known but unavailable sources of evidence.

Search results will be exported to EndNote X9TM for storage and to RAYYAN ^[25] for screening against eligibility criteria.

Evidence screening and selection

Level 1 screening (title and abstract screening)

Pairs of researchers will independently screen titles and abstracts for inclusion according to the pre-determined eligibility criteria. A single failed eligibility criterion will be considered sufficient to exclude a study from this review. Discrepancies will be resolved by discussion between researchers in a pair. In cases where disagreements cannot be resolved, a final decision on the discrepancy will be made by a third party.

The screening process will be pilot tested on a random sample of 50 titles and abstracts.

Level 2 screening (full text screening)

Pairs of researchers will independently screen all located full text articles for inclusion into this review according to eligibility criteria. A single failed eligibility criterion will be considered sufficient to exclude a study from this review. Discrepancies will be resolved by discussion between researchers in a pair. In cases where situations disagreements cannot be resolved, a final decision on the discrepancy will be made by a third party.

This level of the screening process will be pilot tested on a random sample of 10 articles if available.

A chart of the screening and selection process detailing the flow of studies from the search to data extraction and including duplicate removal will be presented with the findings.

An appendix of excluded full -text articles will also be included along with reasons for exclusion.

Data extraction

A data extraction form will be developed *a priori* in Microsoft Excel (2016). The form will capture the following data:

- Study data including authorship, year of publication, article type/study design, country of origin, setting and study objective.
- Participant data including sample size, age, gender.
- Study concept data, that is, reported clinical signs, symptoms and biomarkers of biofilm in chronic wounds.

Data will be extracted by a single researcher and verified by a second.

Critical appraisal/risk of bias assessment

The aim of this review is to collate a comprehensive list of signs, symptoms and biomarkers used to indicate presence of biofilm in chronic wounds regardless of their level of refinement. Relevant data may be found in articles that span the evidence hierarchy and range from systematic reviews to opinion/editorial articles ^[21]. For these reasons, risk of bias assessment of included articles will not be necessary.

Data analysis, summary and presentation

Extracted data will be tabulated. Concept data will be presented in terms of country of origin, setting, study design, and in terms of participant data, that is, sample size, wound aetiology, age and gender. Data will be analysed with SPSS statistical package version 26. Demographic data will be presented descriptively in terms of mean and standard deviation or median and range.

Discussion

Little work regarding biofilms' impact on chronic wounds involving human subjects has been done and much of our clinical understanding is based on *in vitro* work. Clinician's knowledge of research data and of the importance of biofilms in the management of non-healing chronic wounds is inconsistent ^[17]. This review will for the first time, consolidate those signs, symptoms and biomarkers of biofilm in chronic wounds reported in the literature into one document which may serve to open an avenue for future clinical research in this area.

Dissemination

The findings of this scoping review will be published in a peer-reviewed medical journal. The research team will also regularly update and disseminate project findings to key stakeholders, research colleagues, patient representatives and knowledge users.

Study status

Not yet initiated.

Data availability

No data are associated with this article.

Grant information

This project is funded by the Irish Research Council (IRC) and by the Health Research Board (HRB) Collaborative Doctoral Awards (CDA) programme.

Competing interests

No competing interests to disclose.

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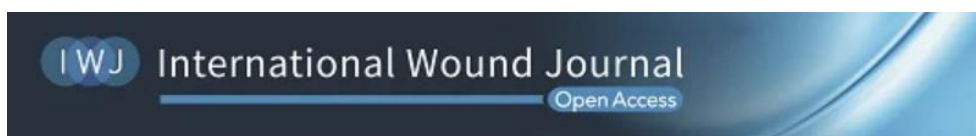
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3.3.3 *Scoping review published results*

This subsection exhibits a report published in the open access ‘International Wound Journal’ (IWJ) that includes the results for this phase of the project (Ivory et al., 2025a).

A characteristics of included studies and a table of excluded studies can be found in Appendix 1.



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REVIEW ARTICLE OPEN ACCESS

A Scoping Review to Identify Clinical Signs, Symptoms and Biomarkers Reported in the Literature to be Indicative of Biofilm in Chronic Wounds.

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Keywords: biofilms | chronic wounds | scoping review | signs and symptoms | wound healing

Abstract

The objective of this review was to identify clinical signs/symptoms reported in the literature to be indicative of biofilm in chronic wounds. The Preferred Reporting Items for Systematic Reviews and Meta-Analysis extension for Scoping Reviews, and the Joanna Briggs Institute Evidence Synthesis manual guided review conduct. Any article/study type reporting signs/symptoms of biofilm in adults with venous, diabetic, pressure and/or mixed arterial/venous ulcers was eligible. Medline, Embase, CINAHL, Cochrane CENTRAL and the Bielefeld Academic Search Engine were searched. Titles/abstracts and full-text articles were screened against eligibility criteria. One-hundred and eleven reports of 109 articles were included. They provided 830 accounts of clinical signs/ symptoms being indicative of biofilm. These were categorised into 26 statements. Visual indicators such as a shiny, slimy layer on a non-healing wound surface quickly reforming in the absence of frequent cleansing or debridement represented 24% of accounts, followed by failed response to antimicrobial therapies (15%), and failure of wound to close or progress to healing despite optimal management strategies (13%). Wound duration >6 weeks and extreme tolerance to host defences represented 1% of accounts. Clinical signs/symptoms are recommended and used as indicators of biofilm presence in chronic wounds but with little supporting validation data.

Summary

- Biofilm has been implicated in the delayed healing of chronic wounds and in the absence of convenient, bedside-friendly diagnostic tests to confirm biofilm presence, clinicians often rely on clinical sign and symptom indicators.

- This scoping review, guided by the Preferred Reporting Items for Systematic Reviews and Meta-Analyses extension for Scoping Reviews (PRISMA-ScR) statement and the Joanna Briggs Institute Manual for Evidence Synthesis, aimed to identify and catalogue which clinical signs and symptoms are being reported in the literature to indicate the presence of biofilm in chronic wounds.
- One-hundred and eleven reports of 109 full-text articles eligible for this review provided 830 accounts of a sign or symptom said to be indicative of biofilm. We refined and categorised these into a list of 26 sign and symptom statements.
- Use of clinical signs and symptoms to confirm presence of biofilm in chronic wounds is commonly recommended, yet very little supporting validation work appears to exist.

1 | Introduction

When the process of wound healing that incorporates phases of haemostasis, inflammation, proliferation and remodelling/maturation becomes disordered or interrupted, injuries to the skin may exhibit delayed or stalled healing and devolve into chronic wounds [1]. These interruptions often stem from comorbidities such as venous insufficiency, arterial disease, diabetes, neuropathies, and unresolved pressure [2, 3]. Genetic factors, radiation and immunological factors can also contribute to chronic wounds [4].

The global prevalence of chronic wounds (mixed aetiologies) has been calculated via meta-analysis to be 2.21 per 1000 population while the pooled prevalence of chronic leg ulcers has been estimated to be 1.5 per 1000 population, and the burdens they cause to patients and healthcare systems in terms of finance and quality of life are well recognized and documented [5-8].

Microorganisms are present in all wounds in what can initially be a host-microbe relationship with no host reaction or delay in healing observed. Whether this initial presence develops along a continuum of colonisation and local wound infection through to spreading and systemic infection is contingent on factors such as increasing bacterial burden, the species of microbes present and inter-species synergy, and the robustness of the host immune system [9].

If not diagnosed and treated promptly, infections in chronic wounds can lead to delayed healing, imposed limits on physical, social and psychological functioning, and a prolonged clinical condition may result [10, 11]. Complications secondary to infected pressure ulcers include endocarditis and meningitis [12], and for persons with diabetic foot ulceration, the risk of hospitalisation increases 50-fold if a wound becomes infected and that of lower-extremity

amputation increases by a factor of 150 [13]. Treatment strategies for chronic wound infections include debridement to remove debris and necrotic tissue along with appropriate use of topical antiseptics and/or systemic antibiotics, but with rates of antibiotic resistance being directly related to level of antibiotic use, clinicians are recommended to apply principals of antimicrobial stewardship to patient care [14].

Biofilms are composed of sessile bacterial cells existing as mono or multi-species communities encased in a self-produced exopolymeric substance that affix themselves to biotic or abiotic surfaces [15]. They are morphologically and physiologically distinct from the planktonic phenotype and are thought to be the prevailing microbial lifestyle in most natural habitats [16, 17]. Biofilms are estimated to be responsible for up to 80% of all human infections [18]. They are thought to be present in 6% of acute wounds but can be found in 60 to 78% of chronic wounds, possibly in over 90% [19-21]. They engage in multiple complex survival strategies that are both inherent to the biofilm phenotype and a result of biofilm-host interactions [22], and after controlling for factors such as ensuring adequate compression, restoring arterial inflow, offloading and management of underlying systemic disease, they may be “the most important single cause of persistent, delayed healing” [23].

Biofilms can be difficult to diagnose and treat, and strategies for both are not without controversy and uncertainty [24]. Microbial culturing is the standard method for identifying pathogenic organisms colonising wounds in clinical settings, but it can underestimate the degree of bacterial colonisation and number of species present in a wound in terms of the biofilm phenotype [25, 26]. This may be due to presence of viable cells in a biofilm that exist in a non-culturable state, that is, they may be dormant or slow-growing variants that do not form colonies under laboratory culture conditions, or biofilms located in deeper tissue [25-27]. Advanced microscopy is an alternative option but is not freely available [27]. Clinicians can rely on clinical signs and symptoms to indicate biofilm presence in chronic wounds in the absence of practical and convenient diagnostic tests [23], but we have not encountered extensive attempts to validate them or collate those reported in the literature into a definitive list.

When thinking about treating or managing biofilm, we ask that if there is uncertainty around its diagnosis and we are relying on unvalidated criteria for confirmatory purposes at the bedside, how do we know that it has truly been removed, and can we be positive that it was there in the first place?

To our knowledge a structured review to map the literature and collate a comprehensive list of signs and symptoms currently used to indicate the presence of biofilm in chronic wounds has

not been undertaken. This review is not an attempt to determine that the signs and symptoms we encounter are those we should be seeking to confirm biofilm presence, rather it is an attempt to address a gap by identifying and cataloguing which signs, symptoms and/or biomarkers within the literature are reported to indicate the presence of biofilm in chronic wounds.

2 | Materials and Methods

2.1 | Design

A scoping review methodology was used to identify signs and symptoms reported in the literature to be indicative of biofilm in chronic wounds. The Preferred Reporting Items for Systematic Reviews and Meta-Analyses extension for Scoping Reviews (PRISMA-ScR) statement and the Joanna Briggs Institute Manual for Evidence Synthesis guided the work [28, 29].

A protocol for this review has been published on the HRB Open Research Website [30], and the key stages are summarised below.

2.2 | Search Strategy and Information Sources

Using keywords and controlled vocabulary identified from articles in the authors' possession and from articles retrieved through preliminary searches of Ovid Medline and EBSCO CINAHL, a search strategy was developed in Ovid Medline [30], peer reviewed according to Peer Review of Electronic Search Strategies (PRESS) guidelines [31] and run in Ovid Medline, Ovid Medline Daily, Ovid Medline In-Process and Other Non-Indexed Citations, Ovid Medline ePub Ahead of Print, Embase, CINAHL and Cochrane CENTRAL. The Bielefeld Academic Search Engine (BASE) was searched for relevant grey literature. The literature search was completed on July 06th 2023. No limits were applied, and all databases were searched from inception to the date on which searches were run.

Reference lists of a random sample of 10% of eligible reports included in the review were scanned, contributions were made by a content expert, and authors of included material were contacted to provide more information where necessary.

2.3 | Eligibility Criteria

2.3.1 | Inclusion Criteria

Articles considered eligible for this review included but were not limited to systematic reviews, randomised controlled trials (RCTs), controlled clinical trials, cohort studies, case-controlled

studies, case series and case reports. Also deemed eligible were letters to the editor with relevant data and editorials.

Articles had to include or refer to adult patients (18+ years) with venous leg ulcers (VLU), diabetic foot ulcers (DFU), pressure ulcers (PU) and/or mixed arterial/venous leg ulcers (MAVLU) treated in any setting, and report on clinical signs, symptoms and/or biomarkers validated or otherwise thought to be associated with the presence of biofilm in chronic wounds.

2.3.2 | Exclusion Criteria

Articles exclusively including or referring to patients with wounds resulting from burns, malignant fungating wounds, wounds secondary to conditions such as rheumatoid arthritis or pyoderma gangrenosum. These wounds have a unique aetiology and physiology that differentiates them from the 'typical' wound for example, VLU, DFU, PU, MAVLU and as such they need to be managed differently.

2.4 | Selection of Evidence

Search results were exported to EndNote X9, deduplicated and then transferred to the Rayyan on-line screening tool [32], where they were deduplicated for a second time.

2.4.1 | Level 1 Screening (Title and Abstract Screening)

A pilot screening exercise was conducted on a sample of 50 titles and abstracts. A follow-up meeting to resolve discrepancies was held and following the meeting all reviewers voted to proceed with the full screening exercise.

Reviewer pairs (J.D.I. & G.G.; J.D.I. & L.P.; J.D.I. & A.V.; J.D.I. & P.C.; J.D.I. & D.S.; J.D.I. & C.H.; J.D.I. & C.F.) independently screened titles and abstracts for inclusion into the review against the pre-determined eligibility criteria. A single failed criterion was considered sufficient to exclude a study. Discrepancies were resolved by discussion between authors in a pair with referral to a third party for a final decision where necessary.

2.4.2 | Level 2 Screening (Full Text Screening)

Full text articles of titles and abstracts retained after title and abstract screening were located by JDI with additional assistance from the University of Galway's James Hardiman Library Interlibrary Loan Service where necessary.

A pilot screening exercise was conducted on a sample of 10 full text articles. A follow-up meeting was held to resolve discrepancies and following the meeting all reviewers voted to proceed with the screening exercise proper.

Pairs of authors (J.D.I. & G.G.; J.D.I. & L.P.; J.D.I. & A.V.; J.D.I. & C.H.; J.D.I. & D.S.; J.D.I. & C.F.) independently screened full text articles against the pre-determined eligibility criteria. Again, an article could be excluded based on a single failed eligibility criterion. Discrepancies were resolved by discussion between authors in a pair with referral to a third party where necessary.

Full text articles located through reference scanning were screened for eligibility by a single review author (JDI).

2.5 | Data Charting Process and Data Items

A data extraction form was developed a priori in Microsoft Excel (2016). The form was designed to capture information including but not limited to study characteristics (author, year, study design/article type, country of origin); patient characteristics (age, sex, ethnicity), wound characteristics (aetiology, dimensions, and duration), and reported signs and symptoms/biomarkers of biofilm in chronic wounds.

Data was extracted by a single study author (JDI) and the extracted data from 21(19%) randomly chosen included articles was verified by a second party (RJ). In the event of a discrepancy, resolution required discussion between both parties with the option to defer to the opinion of a third party if necessary.

For research articles, for example randomised controlled trials or cohort studies, eligible concept data was only extracted from methods and results sections, for other article types, for example literature reviews or editorials, concept data could be extracted from any section.

Characteristics of included studies were presented in tabular form and graphically or textually as frequency of included reports by publication year, country of origin, study design/article type and setting.

Extracted concept data (signs/symptoms reported in the literature to be indicative of biofilm in chronic wounds) was reviewed by study authors and categorised using an inductive content analysis methodology.

The categorised concept data was presented graphically and in tabular form as frequency counts in terms of overall occurrence and occurrence by study design/article type.

Descriptive statistics (mean, standard deviation (SD) and median, interquartile range (IQR) and range) were incorporated with concept data where appropriate.

3 | Results

3.1 | Search Results

The search strategy returned 7724 records. Post deduplication, 4754 titles and abstracts were screened for eligibility (Figure 1). Two-hundred and seventy-eight were passed up for full-text screening and of these, 12 were duplicates, 16 were irretrievable and 148 failed eligibility criteria (provided no concept data [reported signs and/or symptoms of biofilm in chronic wounds] or had an ineligible population)

Reference scanning and contributions from content experts provided 25 full text articles (Figure 1). Four of these were irretrievable and 12 did not meet the review eligibility criteria (provided no concept data or had an ineligible population).

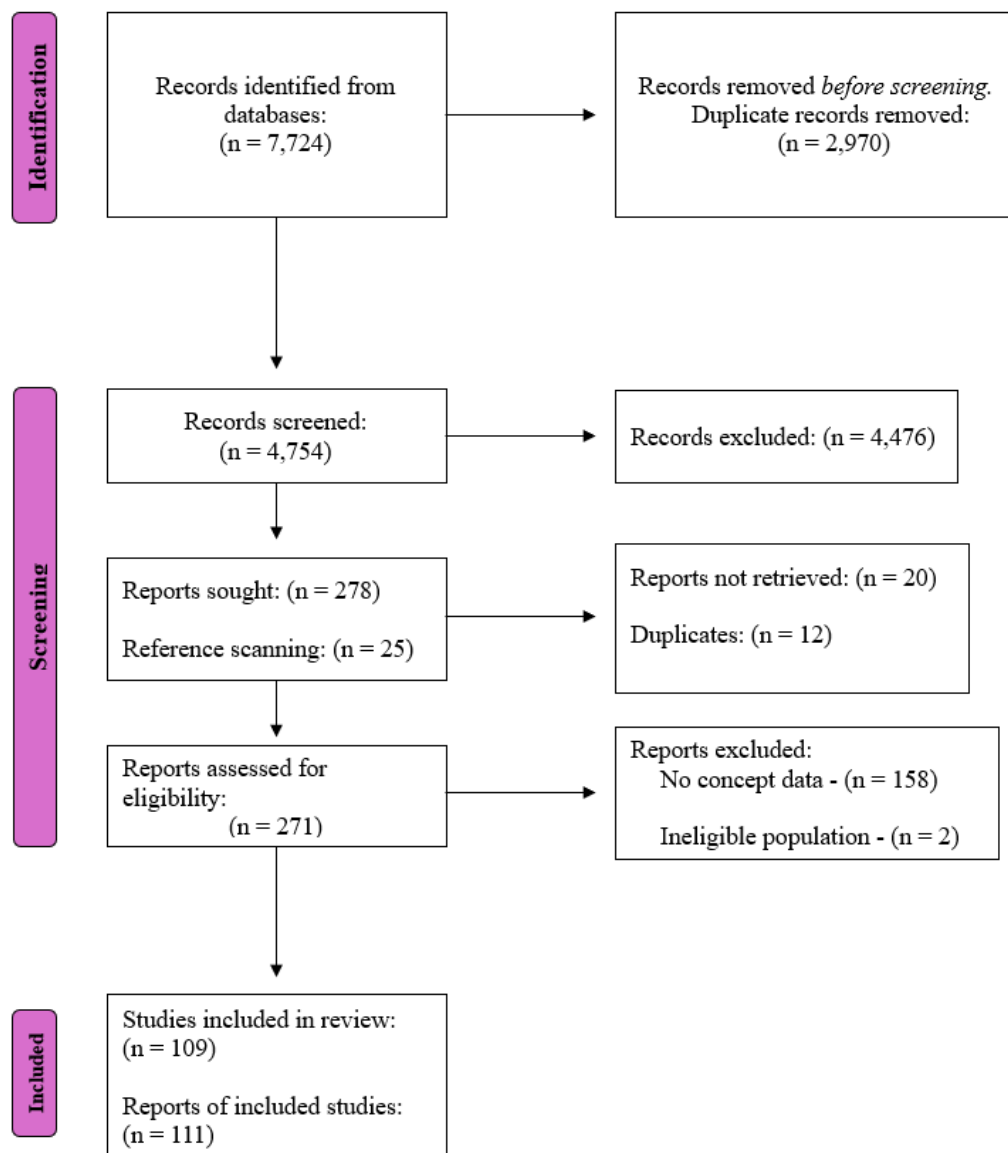


Figure 1 | PRISMA flow diagram of search results (inception to July 6th, 2023 ^[47]).

3.2 | Characteristics of Included Studies

One-hundred and eleven reports of 109 primary articles were included in this review.

The majority of eligible reports were published between 2011 and 2020 while almost none appeared prior to 2006 (Figure 2).

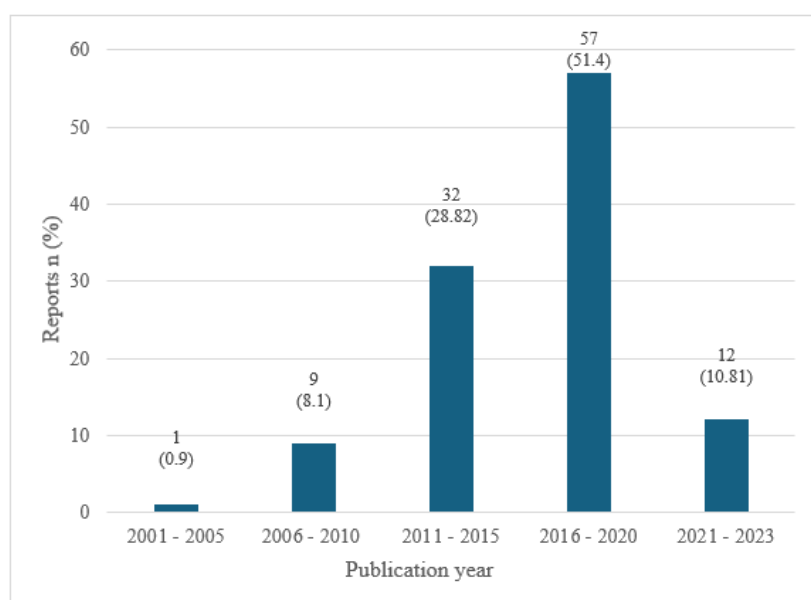


Figure 2 | Included reports n(%) by year of publication.

More than 20 countries were represented with 41 (37%) reports originating from the United Kingdom (UK) followed by 14 (12.6%) from Australia, 14 (12.6%) from the United States of America (USA) and six (5.4%) coming from Spain. Canada, Portugal, China, Colombia, Brazil, Denmark, Germany, and Italy provided between two (1.8%) and five (4.5%) reports each, while Austria, Belgium, India, Ireland, Japan, Mexico, The Netherlands, Poland, South Africa, Switzerland and Taiwan each provided one (0.9%) report.

In terms of study design/report type, the majority were classed as literature review type articles with no elements of systematic review methodology reported. Two of these had an associated case series and one an associated case report. The least common report types were position documents, practice recommendations, cohort studies and scoping reviews of which one each (0.9%) were included (Figure 3).

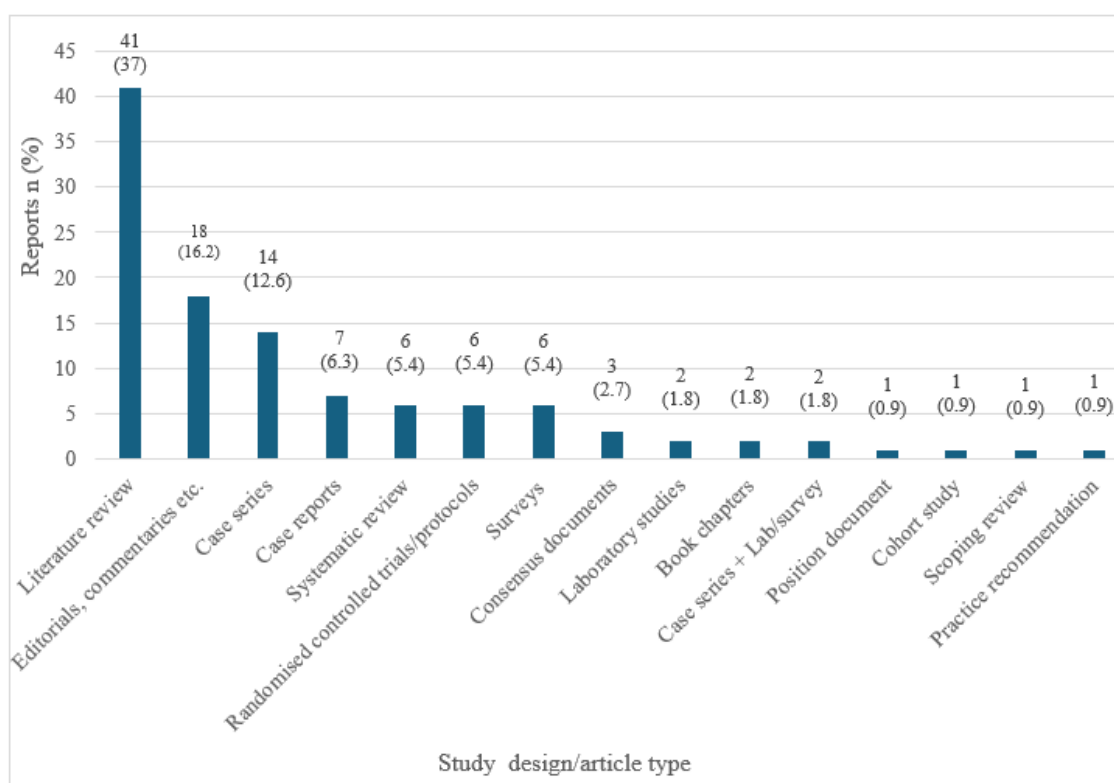


Figure 3 | Included reports n (%) by study design/article type.

The setting was recorded in 31 (28%) reports. Dedicated wound care clinics were the setting of choice in 11 (10%) reports followed by a virtual setting in 6 (5.41%). Five (4.5%) studies took place in a hospital setting and two (1.8%) recorded a routine care setting/public health centre. Seven (6.31%) took place in undefined healthcare facilities or specified hospital departments. Setting was not reported or did not apply in 80 (72%) reports.

Diabetic foot ulcers were the most common aetiology. Sixty-nine (62.3%) reports did not specify an aetiology and typically made reference to “chronic wounds”, “complex wounds”, “hard-to-heal wounds” and so forth (Figure 4). Patient and wound demographic data were recorded inconsistently and appeared in 24% and 16% respectively of the included reports. This data is not representative of the whole sample of included articles and is not presented in this review.

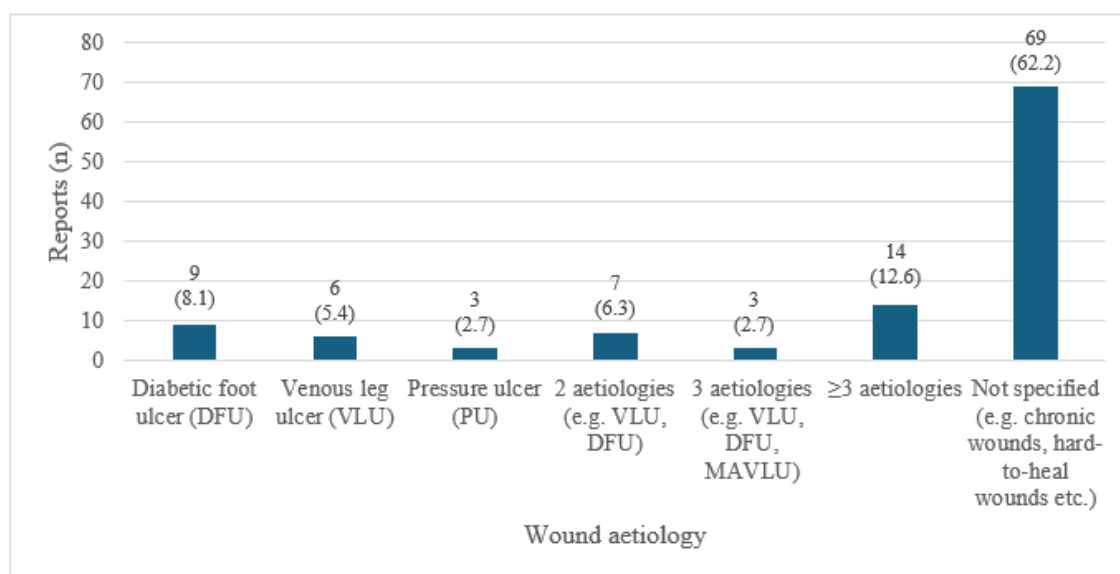


Figure 4 | Included reports n (%) by wound aetiology.

3.3 | Reported signs and symptoms of biofilm in chronic wounds

Signs and symptoms reported in the literature as being indicative of biofilm were encountered 830 times in the 111 included reports. The average number of signs and symptoms per included report was 7.4, SD 8.8 (median five, IQR five). The range was from 1-51.

In terms of study design, literature reviews produced 214 (26%), case series produced 204 (24.6%) and surveys produced 93 (11.2%) reported signs and symptoms. The least number of signs and symptoms produced was one (0.12%) and this came from a cohort study design (Figure 5).

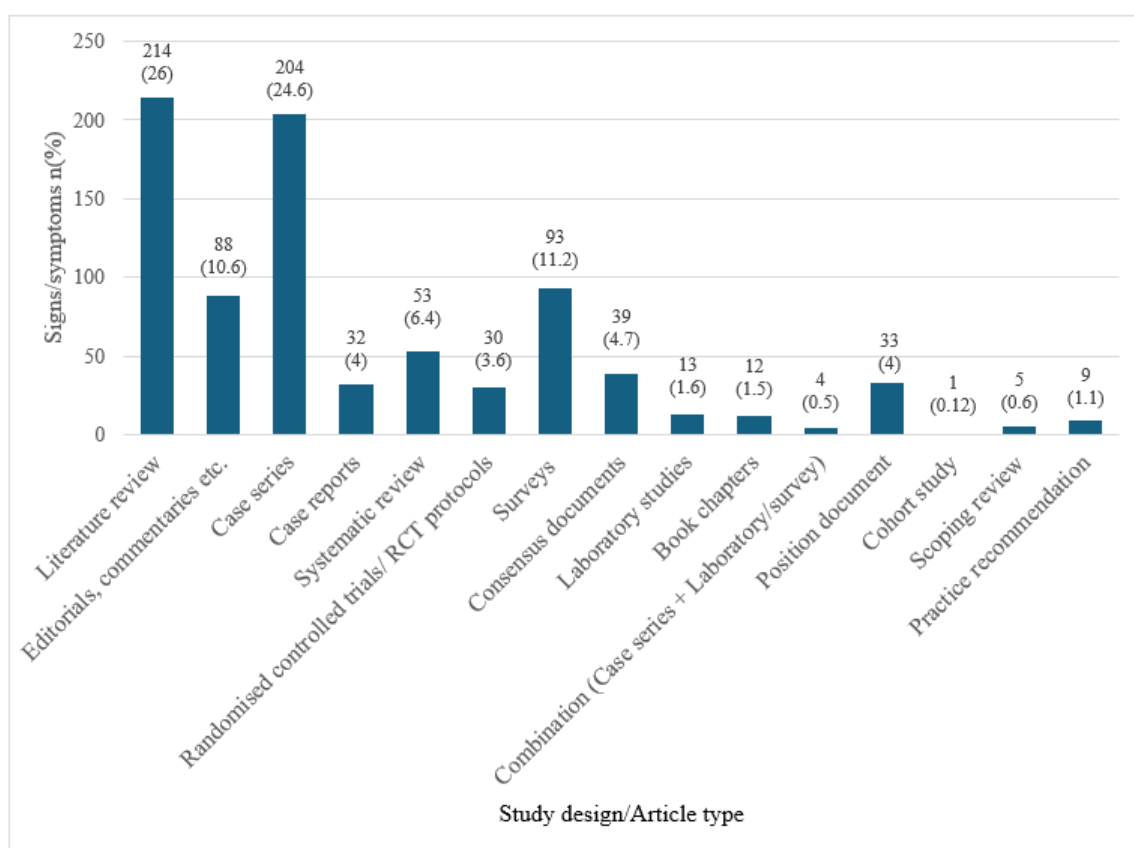


Figure 5 | Signs and symptoms reported to be indicative of biofilm in chronic wounds n (%) by study design/article type.

Within the included articles, signs and symptoms were reported in different ways using different descriptions and terminologies. References to delayed healing included for example “Indolent wound despite healing”, “wound is not healing”, “delayed healing” or “stalled wound”, and references to exudate included “copious exudate”, “exudating”, or “excessive exudate”. Following discussion and review among co-authors they were grouped into a series of 26 more comprehensive and succinct sign/symptom statements (Table 1). Visual indicators were reported most frequently and were encountered 198 (24%) times followed by failure to respond as expected to antimicrobial therapies which occurred 125 (15%) times and failure of a wound to progress to healing which occurred 108 (13%) times. Wound duration of more than 6 weeks and extreme tolerance to host defences were encountered only one time (0.9%) each across all included articles.

Table 1 | Refined and categorised signs and symptoms reported in the literature to be indicative of biofilm in chronic wounds.

Sign/symptom	Occurrence in literature n (%)
Visual indicators: A shiny, slimy layer on a non-healing wound surface that persists despite autolytic or enzymatic debridement but can be removed easily with physical removal techniques such as cleansing or mechanical debridement. The surface reforms quickly (1-2 days) in the absence of frequent intervention e.g. cleansing, debridement.	198 (24)
Failure to respond as expected to antimicrobial therapies e.g. systemic antibiotics and/or topical antimicrobials/antiseptics. Infection may re-emerge when treatment is completed.	125 (15.1)
Wound fails to close or progress to healing despite optimal wound/patient management.	108 (13.01)
Increased exudate.	63 (7.6)
Presence of slough or fibrin.	50 (6)
Signs of local infection e.g. erythema, oedema, serous effusion, new areas of breakdown, pain, etc.	45 (5.42)
Persistent or prolonged (low-level) inflammation.	43 (5.2)
Poor quality granulation tissue e.g. discoloured, friable, fragile or hypergranulation.	42 (5.1)
Signs of necrotic tissue/eschar.	28 (3.4)
Infection lasting more than 30 days or persistent cycles of recurrence (waxing and waning).	23 (2.8)
Favourable response to therapies such as corticosteroids, TNF α inhibitors, or multimodal strategies such as physical debridement, cleansing and topical antimicrobial agents and dressings.	21 (2.5)
Malodour.	20 (2.41)
Negative cultures despite signs/suspicion of clinical infection.	15 (1.81)
Wound pain/pain at site.	12 (1.5)
Low-level/increased erythema.	10 (1.2)

Wound edge e.g. maceration, non-physiological colour changes of the wound margin, hyperkeratosis.	4 (0.5)
Swelling/wound bed with pale oedema.	4 (0.5)
Debris or foreign objects in the wound e.g. pins, mesh, prosthetic devices.	4 (0.5)
Soft tissue deterioration despite antibiotic treatment and/or surgical debridement.	4 (0.5)
Tunnelling (along tissue structures, degradation along solid structures such as tendon or bone) and/or undermining (erosion of the subcutaneous layer under the wound edge).	3 (0.4)
Temperature. Sometimes low-grade fever.	2 (0.24)
History of systemic antibiotics in the last 12 months, prior or current use of antimicrobials.	2 (0.24)
Wound duration of more than 6 weeks.	1 (0.12)
Extreme tolerance to host immune defence.	1 (0.12)
Sepsis	1 (0.12)
Other patient history indicators e.g. change in the patient's overall wellbeing or quality of life.	1 (0.12)

4 | Discussion

Currently, there are no clear-cut or routine tests available for use at the bedside to help clinicians determine if biofilm is present in chronic wounds [23, 25]. A study published in September of this year involving 40 patients with chronic wounds demonstrated that a fluorescence imaging device and a wound blotting technique had sensitivities of 84% and 24% respectively, specificities of 27% and 64%, and accuracies of 63% and 40% for detecting biofilm when compared against scanning electron microscopy (SEM) and polymerase chain reaction (PCR) microbiology gold standards [33]. The most reliable approaches include advanced microscopy techniques that are not intended for use in bedside practice [23, 24, 34]. Overt or classic (traditionally associated with local infection) signs and symptoms such as erythema, warmth, swelling and malodour, or covert (subtle, typically manifesting before the onset of overt indications) signs and symptoms such as hypergranulation, increasing exudate and delayed healing are routinely used to diagnose infections in chronic wounds [35], and given the diagnostic issues described above it is not surprising that a similar approach would be applied to determining presence of biofilm. Indeed, the statement “In the absence of bedside diagnostic

tests, specific signs and symptoms should be used to confirm biofilm presence.” received strong agreement in a 2017 consensus guideline although it was not a key statement [23].

This scoping review included 111 reports that yielded 830 accounts of a clinical sign or symptom thought to be indicative of biofilm in chronic wounds. Some reports provide a mixture of visual cues and other clinical signs. Metcalf et al. for example refer to “viscous, slimy or gel-like substances that can form on, beneath or even within some dressings”, excessive moisture and poor quality granulation tissue [36]. Bowlby & Blume write that “the presence of slough, shininess to an ulcer, slough or necrotic tissue is likely an indication that biofilm is present” [37]. Other authors tend to emphasise the visual. Hurlow et al. describe a “cloudy, shiny, thin slime material on the ulcer bed that persisted despite daily cleaning with pulsed lavage”, and “a cloudy, translucent film on the wound surface through which larger granular buds protruded” in their 2009 case series [38]. Still others describe only clinical cues. Haesler and Ousey write that chronic wounds exhibiting delayed healing despite optimal conditions, recalcitrance to antimicrobial therapy and antibiotic failure may have biofilm present. In addition, they refer to signs of early local infection such as increasing exudate and moisture, low level inflammation and erythema, and poor granulation or friable hypergranulation [39].

However, while it is important to recognize symptoms of wound biofilm, clinical diagnosis can be subjective [40]. It is also acknowledged that wound biofilms are difficult to visualize macroscopically, with the possibility of mistaking slough, debris and exudate for biofilm [23]. In addition, slough in a wound and other visual markers of biofilm have been deemed problematic and seen as being too subjective, without basis and objective criteria [40]. Interestingly, visual cues were the most encountered sign of biofilm in this review. The frequency of reporting of a sign or symptom thought to be indicative of biofilm does not necessarily reflect its validity, that is, a sign/symptom reported 20 times holds no more importance in terms of validity than one reported two or three times. Indeed, of the included reports in this review, only two provided validation data in relation to signs and symptoms of biofilm in chronic wounds [41, 42]. Using histology (haematoxylin and eosin staining) and SEM, Hurlow et al. identified biofilm in 12/16 (75%) of samples of a “firmly adherent, gel-like wound bed film which had reformed under antiseptic wound dressings and sometimes despite guided systemic antibiotics” taken from 11 consenting participants with chronic non healing wounds [41].

As a secondary objective in their microscopy visualisation study, Johani et al. [42] set out to correlate six clinical wound observations (presence of slough, excess exudate, poor tissue

quality, signs of pyocyanin, gelatinous wound surface and gelatin reforming quickly) against microscopy (peptide nucleic acid fluorescent in situ hybridisation [PNA-FISH] confocal laser scanning microscopy [CLSM]) of biopsies taken from 26 diabetic foot ulcers. They found that apart from excessive exudate, the probability of them facilitating clinicians in identifying wound biofilm was no better than chance alone. They proposed that clinical cues are not useful for detecting biofilm presence in DFUs, but that larger sample sizes and other chronic wound aetiologies would be required to verify their results. Subsequent to this review, a study from 2024 reported the sensitivity, specificity and accuracy of clinical signs of biofilm to be 44%, 40% and 43% respectively when compared against SEM and PCR microbiology gold standards. However, it is not clear from the study how these signs were assessed, that is, as single entities or as aggregates/combinations [33].

Solutions to the biofilm diagnosis problem in chronic wounds could perhaps take the form of making microscopy methodologies a more routine part of clinical/laboratory practice. More realistically, we could develop bedside diagnostic devices, indeed there are novel technologies currently in existence such as wound blotting and point-of-care fluorescence detection [34, 43, 44]. Another avenue could be to work with clinical indicators and seek to further determine their diagnostic capabilities through validation work as has been done in the case of acute foot and ankle injuries and systemic lupus erythematosus (SLE) [45, 46].

4.1 | Limitations

Due to time and resource restrictions, rather than have 2 reviewers independently extract data, one reviewer extracted data, and this was verified in 21(19%) of the included articles by a second party. However, the sample for verification was chosen randomly and the agreement rate in that sample was almost 100%.

Reference scanning returned nine eligible full texts, and it is possible that had the reference lists of all included articles been scanned rather than a percentage, more eligible articles could have been returned. Nevertheless, given that the purpose of this review was to collate a list of signs and symptoms reported in the literature to be indicative of biofilm in chronic wounds, and that extracted data was repetitious in nature with the same signs and symptoms appearing time and time again, it is unlikely that any new or unexpected data would have been retrieved. For the same reasons it was thought unlikely that extensive efforts to contact content experts would add anything new to the review. This review was concerned more with producing a comprehensive list of signs and symptoms than with documenting the exact number reported in the literature and so the methodology as it stands was adequate to achieve this.

Finally, the topic of interest for this review is not typically reported as primary or secondary outcomes data that would appear in an article abstract and so it is possible that eligible articles were missed in the title and abstract screening phase. It would not have been feasible to locate and screen full texts of all titles and abstracts, but our search strategy was comprehensive. Four literature databases and one grey literature source were searched with an extensive strategy from inception to date of search without any limits, and the search was subsequently updated. In addition, as stated above, observing the same signs and symptoms appear repeatedly lead us to conclude that no significant data was missed.

5 | Conclusion

It would seem apparent that in terms of using visual cues and clinical signs and symptoms to determine presence of biofilm in chronic wounds, the territory is not very clearly mapped out. The concept is commonly recommended and employed but is not without its critics. This review recovered numerous accounts of signs and symptoms which we refined into a series of statements deemed to be indicative of biofilm in chronic wounds, but it only uncovered validation work on four of these (presence of exudate, slough, poor tissue quality and a quickly returning gelatinous surface), the results of which are not particularly supportive of the idea. It could be argued that confirming presence of biofilm in chronic wounds is not necessary, that we are over-complicating matters and that it would be more beneficial in terms of patient outcomes and resource use were we just to focus on debriding away devitalised tissue, biofilm and other debris to promote wound healing. However, it is generally accepted that biofilm contributes to delayed healing, and we are unaware of any new evidence that would ask us to reconsider that consensus. Indeed, a commonly reported indicator in the literature refers to delayed healing *despite optimal management*. Furthermore, global expenditure on biofilm-related complications in chronic wounds is high and there are many treatment strategies that claim to completely remove biofilm, but their supporting evidence largely comes from *in vitro* work which demonstrates efficacy but not necessarily real-life effectiveness.

We would therefore advocate that in the interests of optimising patient care and streamlining treatment strategies, a concerted effort to validate or nullify the reported signs and symptoms of biofilm in the literature be carried out and the findings of this scoping review could serve as an ideal starting point.

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Conflicts of interest

The authors declare no conflicts of interest.

Data availability statement

The data that support the findings of this study are available from the corresponding author upon reasonable request.

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Supporting information

Additional supporting information can be found online in the Supporting information section

3.3.4 Scoping review results ex publication

Thirty-one of the reports (17 case series, seven case reports, four randomised controlled trials, two laboratory studies and one cohort study) included a total of 720 patients. Mean age was reported in 23 of these (491 patients) and was calculated to be 66 years overall. Sex was recorded in 23 reports (482 patients) and 43.32% of these were female. Mean wound duration was recorded in 11 studies (112 patients) and was calculated to be 25.3 months. Finally, mean wound dimensions were recorded in 9 reports (86 patients) and was calculated to be 21cm².

3.4 Thesis phase 2: An electronic Delphi consensus study

3.4.1 Introduction

According to the World Health Organisation (WHO), point-of-care (POC) diagnostics should meet the criteria specified in the REASSURED (Real-time connectivity, Ease of specimen collection, Affordable, Sensitive, Specific, User-friendly, Rapid and Robust, Equipment free or simple Environmentally friendly, Deliverable to end-users) framework (Otoo and Schlappi, 2022). Any prototype diagnostic tool arising from this research work would therefore be anticipated to fit within this framework.

While planning and developing the methodological processes for this project it was not possible to confidently forecast the size of the final list of reported biofilm signs and symptoms that the scoping review phase would generate until that phase was complete. An extensive list would be unwieldy and cumbersome to manage in a clinical assessment scenario and so it was concluded that a means of refining it to a more user-friendly size would be necessary. In addition, an element of clinical expertise/opinion incorporated into the development process was desirable and that priority be given to those signs and symptoms determined by clinical personnel to be most likely indicative of biofilm in chronic wounds in the final analysis phase. Consensus is defined in the Merriam-Webster dictionary as ‘general agreement’ or ‘unanimity’ (Merriam-Webster, 2025). Rulkens et al. wrote that it can occasionally be unclear whether consensus exists on a subject at a given point in time, but that expert consensus methods can mitigate this uncertainty by ‘intentionally and formally pursuing or assessing consensus’ (Rulkens et al., 2024). Methods of determining consensus include brainstorming, nominal group technique, consensus conference, Glaser’s state of the art method and the Delphi technique. A brief description of each method will now be provided followed by justification for choosing the Delphi method for this phase of the project.

Brainstorming is ‘an informal process of using a diverse group of people to get at the root causes of problems’ and involves steps of stating the problem or objective, setting ground rules, allowing all to participate, soliciting ideas and presenting them visually, eliminating irrelevant or duplicative ideas and reaching consensus on ideas with the highest priority (Evans). A system of voting may be required and the process typically involves six to 12 individuals participating in a physical setting, preferably neutral with no distractions (Evans). It is considered to be more appropriate in scenarios where there are many potential solutions to a problem and complex issues need to be addressed in a fast and informal way (Evans).

The nominal group technique ‘is a structured method of group decision-making which allows a rich generation of original ideas, balanced participation of all members of the group, and a rank-ordered set of decisions based on a mathematical voting method’ (Horton, 1980). This technique requires that the matter of concern or Nominal Question be presented to a group of seven to ten individuals in a round-table scenario. Group members take five to ten minutes to silently note their answers to or thoughts on the Nominal Question before sharing them with the other members. Ideas are recorded, presented to the group and a round of free discussion on each idea then follows where further ideas may be generated, clarification sought and provided, and opinions may be expressed. After a period of silent consideration, members are finally asked to score presented ideas in terms of relative merit or importance (Horton, 1980, Rulkens et al., 2024).

A Consensus conference is a ‘formal public meeting, which gives the general public the chance to contribute to and be involved in the assessment and discussion of views and evidence around an issue or proposal’ (Better Evaluation Knowledge, 2025, Rulkens et al., 2024). In essence it serves to provide a forum in which dialogue between experts on a topic of concern and citizens can take place (Better Evaluation Knowledge, 2025). A panel of citizens derives a series of questions to be addressed at the conference, disseminating them to other concerned citizens and participates in selecting an expert panel needed to answer them (Better Evaluation Knowledge, 2025, Rulkens et al., 2024). Typically this panel is chosen with a view toward ensuring that opposing views and conflicts can emerge and be discussed at the conference (Better Evaluation Knowledge, 2025). The success of this consensus technique depends on strict adherence to rules of implementation overseen by an advisory/planning committee that ensures that the process is fair, democratic and transparent (Better Evaluation Knowledge, 2025). The primary advantages of the consensus conference are that it helps to bridge the gap between experts and the lay public, it demonstrates plurality in terms of views on a topic of concern, and it facilitates public debate from a range of perspectives. Conversely, the costs involved can be high (Better Evaluation Knowledge, 2025).

Glaser’s state of the art technique is a ‘levelled approach to obtaining consensus’ in which a selected facilitator convenes a core group or project team of experts in a field (Rulkens et al., 2024, Glaser, 1980). Through an iterative process the core group drafts a position paper on a topic of interest which gets circulated to a wider expert group for critical appraisal. A draft then goes to a group of independent reviewers through a journal specialising in the topic of interest. The draft goes through waves of revisions whereby modifications suggested by invited

reviewers are incorporated, the modified draft is sent to more reviewers, and the process repeats until a point of diminishing returns when the paper gets published by the journal' (Rulkens et al., 2024).

The Delphi technique is a structured, iterative process that uses a series of sequential questionnaires or rounds in an attempt to achieve convergence of opinion into consensus among a group of experts in a field on a topic of interest that can be administered electronically (Keeney et al., 2001, Williamson et al., 2017). Experts may come from diverse backgrounds and geographical locations, and their participation (in large numbers if necessary) is facilitated through four methodological features (Keeney et al., 2001, Williamson et al., 2017): 1) The experts or panellists are questioned on the topic of interest and typically asked to rate items on the topic on scales such as a 9-point Likert. 2) The process is anonymous which helps mitigate complicating factors such as social pressure and conformity to a dominant view. 3) The process is iterative and incorporates multiple (traditionally 4) rounds of questionnaires in a continuous process until consensus is reached. 4) Subsequent rounds in the process are informed with feedback which allows different perspectives to be considered prior to re-rating. At the end of each round, panellist responses are aggregated and presented in the next with descriptive statistics. Panellists may also be asked to explain their rating choices and these explanations may also be included with feedback (Keeney et al., 2001, Williamson et al., 2017, Jünger et al., 2017).

Advantageous features of the Delphi technique include its ability to bring together participants from geographically diverse areas, inherent anonymity, controlled feedback provided between rounds, and flexibility in terms of choosing statistical analysis methods (Shang, 2023). Conversely, the Delphi method has been critiqued for reasons including having multiple versions that lack uniformity in their methodological approaches, leading to questions in terms of reliability and validity of findings (Keeney et al., 2001). In addition there are perceived problems around the use of 'experts' in that expert opinion in the context of Delphi is 'scientifically untenable and overstated', the term can be potentially misleading because having knowledge of a particular topic does not make one an expert and there is potential for selection bias as the composition of a panel can affect findings (Keeney et al., 2001).

Nevertheless, given that the consensus methods described above are for the most part designed to accommodate small sample sizes, require a single physical location where anonymity is impossible, or are unwieldy and time consuming, the Delphi technique with its ability to bring together potentially large numbers of individuals from diverse geographical areas in a non-

local, anonymous fashion was therefore considered to be the most appropriate methodological approach for this phase of the project.

3.4.2 *Electronic Delphi published protocol*

The following is a protocol for this phase of the project, and it has been published in the European Wound Management Association's (EWMA) 'Journal of Wound Management' (JWM) (Ivory et al., 2023).



Signs, symptoms and/or biomarkers reported to indicate biofilm in chronic wounds: an eDelphi consensus protocol

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Keywords: biofilm, Chronic wounds, signs, symptoms, eDelphi, consensus

For referencing

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Abstract

Background: Chronic wounds secondary to conditions including venous insufficiency, diabetes or unrelieved friction/shear are a significant burden to patients and healthcare systems. Biofilms are estimated to occupy 60-78% of these wounds. However, they are difficult to detect macroscopically and have no definitive clinical markers. Clinicians are thus advised to assume biofilm presence in all stalled wounds. Incorrect diagnosis of biofilm presence may result in sub-optimal care provision and inadvertent, inappropriate administration of medical interventions.

Hypothesis/Aim: To achieve clinical consensus on which signs, symptoms and/or biomarkers (items) currently reported in the literature are most likely to indicate presence of biofilm in chronic wounds.

Methods Design, Sample, Data collection, Data analysis: An international steering committee of researchers and wound clinicians convened to develop this protocol for a 2 round electronic Delphi process that will recruit ≥ 30 active clinicians with ≥ 3 years clinical experience, $\geq 50\%$ of which must be in wound care. Participants will rate items on a 9-point Likert scale. Consensus to include items requires $\geq 70\%$ of participants rating 7-9 and $\leq 15\%$ rating 1-3.

Conclusions: Integrity of research findings depends on methodological rigour. Concerns exist regarding consistency of methods in Delphi studies. Given these concerns we decided that review of Delphi methodological dilemmas by a group of experienced clinicians and researchers would be the most appropriate means to optimise the methods for this particular project.

Implications for clinical practice: Findings will consolidate current clinical opinion on what signifies biofilm in chronic wounds. Delphi methodology should be standardised to develop its rigour.

Key messages

- Biofilms, capable of delaying healing and thought to be present in 60-78% of chronic wounds are difficult to detect macroscopically.
- This protocol describes an eDelphi process that aims to gain clinical consensus on which signs, symptoms and/or biomarkers currently reported in the literature are most likely to indicate presence of biofilm in chronic wounds.
- Uniformity and standardisation of Delphi methodology would improve validity and credibility of findings

Introduction

Biofilms are groupings of microbial cells that enclose themselves in a matrix of polysaccharide material and adhere to surfaces such as indwelling medical devices, water system piping or living tissue.⁽¹⁻³⁾ Indeed, it is assumed that 99.9% of the total microbial biomass on earth exists in the biofilm phenotype.⁽²⁾

Chronic wounds are vulnerable to occupation by multiple microbial species presenting in this phenotype with prevalence estimated to range from 60 – 78% and the consensus is that

pathogenic biofilm types contribute to delayed healing.⁽⁴⁻⁶⁾ These pathogens may halt the wound in the inflammatory phase, impair granulation tissue formation and reduce epithelialisation.^(4, 6)

Biofilms can be challenging to detect in a wound at the macroscopic level. There are some features on the wound surface such as extensive fibrinous slough, visible to the naked eye that may be mistaken for biofilm. Additionally, it has been reported that biofilms may not present uniformly across the wound bed and may also be located below the wound surface, although evidence to support this hypothesis is limited.^(5, 7-10) Erroneously diagnosing biofilm may lead to suboptimal provision of care.⁽⁵⁾ Currently, there are no clinical markers that can definitively indicate the presence of biofilm on biotic surfaces and clinicians are advised to assume that all chronic wounds demonstrating delayed healing have biofilm present.^(5, 11) We suspect that this may result in unintentional misuse of antimicrobial agents, adding more cost and increased risk of developing multi-drug resistant bacterial strains without necessarily improving patient outcomes.

The Delphi technique is a structured consensus method, originally developed by the Rand Corporation in the 1950s that is commonly applied in areas such as education, environmental science, management and health care for the purposes of priority determination, problem solving or ideas generation.⁽¹²⁻¹⁴⁾ It has been defined in one instance as “an iterative process designed to combine expert opinion into group consensus”⁽¹⁵⁾ and it permits large numbers of individuals across a wide range of expertise and settings to be included in an anonymous fashion. The technique helps to reduce negative effects of group interactions such as dominance by one or more individuals.⁽¹⁵⁻¹⁸⁾ Consenting participants or panel members engage in multiple rounds of a questionnaire and receive feedback after the closing of each round. This feedback can constitute information in terms of descriptive statistics on how questions were answered by individual participants and/or by the panel and facilitates reconsideration of opinions by participants in the light of the whole group.⁽¹⁴⁻¹⁹⁾ Typically, the process continues until group consensus is reached.⁽¹⁵⁾

This paper reports a protocol for an eDelphi method to gain consensus from an international sample of experienced wound care clinicians/clinician-researchers on which clinical signs, symptoms and/or biomarkers (defined characteristics that are measures as an indicator of normal biological processes, pathogenic process or responses to an exposure or intervention⁽²⁰⁾) that can be measured at the bedside and are currently reported in the literature are the most likely to indicate presence of biofilm in chronic wounds.

Methods

An international steering committee comprised of nine experienced, wound care clinicians and researchers convened to finalise the methodology for this project.

We took eleven areas of Delphi methodology reported in “The COMET Handbook: version 1”⁽¹²⁾ and used them to structure the agenda for this exercise. These areas are: 1. Number of panels; 2. Group size; 3. Participant information; 4. Number of rounds; 5. Questionnaire structure; 6. Scoring methods; 7. Between-round feedback; 8. Criteria for outcome retention; 9. Attrition; 10. Definition of consensus; and 11. Means for assessing consensus.

Sample size

We wish to recruit ≥ 30 clinical personnel, actively engaged in wound care practice including nursing, podiatry and medicine to form a single participant panel for the eDelphi process.⁽²¹⁾
⁽²²⁾ We will incorporate a snowballing sampling technique into the recruitment process, meaning that we cannot predict a final sample size at this point.⁽²¹⁾

Sample size for a Delphi exercise is not decided on the basis of statistical power and it can vary considerably between projects, with for example, anywhere between 12 and >300 participants recruited. Deciding on the number of participants to recruit can often be a matter of practicality.⁽¹²⁾

Finalising sample size and choosing a minimum rather than a maximum number was achieved through discussion between steering committee members that centered around factors of representativeness, best estimate of external validity, attrition, participant appropriateness and practicality, in conjunction with our mixed recruitment strategy (see below).

Eligibility

We will include adults (≥ 18 yo) based on their involvement and experience in wound care clinical practice or wound care research in conjunction with clinical practice.⁽²³⁾

Participants may be from any geographical region worldwide and must possess ≥ 3 years clinical experience, $\geq 50\%$ of which has been spent in a wound care environment. Participants must be experienced and proficient in assessing wounds in accordance with guidance such as the wound bed preparation paradigm⁽²⁴⁾ and have at least basic, self-declared microbiological knowledge of biofilm development, management and control in the wound care context. Eligible participants may or may not have published on the topic of biofilm in chronic wounds. In round one of this exercise, we will capture the following demographic data from consenting participants - gender, age, country of practice, specialty (e.g. nursing, medicine, podiatry etc.),

practice area (e.g. primary care, gerontology, etc.) years of clinical wound care experience and number of relevant publications (if any).⁽²³⁾

We considered four possibilities in regard to appropriate participants for this process: a single participant group of biomedical researchers; a single participant group of clinical personnel; a mixed research/clinical participant group; or two separate clinical and research participant groups. This eDelphi work is concerned more with clinical experience and opinion than academic/laboratory expertise, yet it could also benefit from research input. The steering committee thus decided that the best way to balance both would be to choose clinically experienced participants who may or may not have research experience in this field.

Recruitment strategy

An invitation to participate email will be drafted. This email will contain a link to the online survey, contact details of the research lead and a participant information leaflet describing the nature and background of the study, expectations of participants and their rights and a description of how data will be collected and managed.⁽²⁵⁾

We will identify a list of national and international organisations involving wound care clinicians and contact their gatekeepers with a request to distribute the email invitation to members. We will then ask recipients of the invitation email to forward it to potentially eligible colleagues on their contact lists. We will also seek to recruit participants through our own Alliance for Research and Innovation in Wounds (ARIW) twitter account (@ariw_1) and via social media accounts of steering committee members.

Round one

We will create a questionnaire containing a randomised list of clinical signs and symptoms thought to be indicative of biofilm in chronic wounds (items) derived from a scoping review of the literature.⁽²⁶⁾

Participants will be asked to rate each item on the list along a 9-point Likert scale split into three domains of choice:⁽¹²⁾

- 1 – 3 = Unlikely to be indicative of biofilm in chronic wounds.
- 4 – 6 = Somewhat likely to be indicative of biofilm in chronic wounds.
- 7 – 9 = Very likely to be indicative of biofilm in chronic wounds.

Finally, participants will be asked to provide a brief justification for their scores in a text box associated with each item. This will be an optional step and information provided will be

included as part of the feedback presented to participants at the end of round one (see feedback between rounds below). This should contribute to the comprehensiveness of the feedback and may facilitate convergence of opinion on items presenting in round two.

The questionnaires will not have an open question option as this may introduce an excess of data managed differently from that which has passed through the scoping review process. Additionally, such questions may lead to an influx of unique items that are not encountered in clinic on a regular basis.

The duration of this round will be 21 days maximum. ⁽¹²⁾

Round two

Items with a 'no consensus' decision (see definition of consensus below) at the end of round one will be collated, and participants will be asked to rate each item on the list on a 9-point Likert scale split into three domains of choice as reported for Round 1 above. ⁽¹²⁾

This round will not have an open question option. Participants will not be asked to provide feedback describing the reasons for their decisions at the end of this round. Items with a clear 'excluded' decision will be discarded and items with a clear 'included' or 'no consensus' decisions will be retained (see definition of consensus below).

The duration of this round will be 21 days maximum. ⁽¹²⁾

All items from rounds one and two designated 'include' and items from round two designated 'no consensus' (see definition of consensus below) will be collated into a list of potential signs and symptoms of biofilm in chronic wounds. This list will be subject to testing against clinical samples as part of a biofilm diagnostic project.

Definition of consensus

We will define consensus on each included item on the list as follows: ^(25, 27)

- Include: $\geq 70\%$ of participants rate item 7–9 and $\leq 15\%$ rate item 1–3.
- Exclude: $\geq 70\%$ of participants rate item 1–3 and $\leq 15\%$ rate item 7–9.
- No consensus: Any item not meeting the criteria above.

Only items with a 'no consensus' decision will be carried forward to round two of the process.

⁽²⁸⁾

Feedback between rounds

At the end of round one an analysis of the ratings will be presented in tabular/graphical formats. This will include the group median (IQR) score and percent agreement among participants for each item. Any justifications provided by participants for scores will be summarised into brief statements and added to items intended for circulation in round two. (12, 25, 28)

Assessment of consensus

Williamson et al. recommend that researchers examine the degree of consensus in each round to ensure that the Delphi process is working as intended. They suggest examining changes in individual's scores between rounds. (12)

We will assess consensus in this research by means of a Bland Altman analysis. (29) The Bland Altman method creates statistical limits of agreement using the mean and standard deviation of the differences between two measurements to quantify agreement between two methods of measurement. This is represented by a plot of the difference between paired measurements on the y-axis vs. the mean of both measures on the x-axis. The method recommends that 95% of data points lie within ± 2 standard deviations of the mean difference. Expected limits of agreement will be determined *a priori* and normality of distribution will be determined graphically. If differences are not distributed normally, appropriate transformation will be used to satisfy the normality assumption.

Strategy to maintain response rate

Attrition bias occurs when participants who do not respond in subsequent rounds have different views from peers who continue to participate. If feedback suggests that a participant is in the minority with respect to scoring of particular outcomes, they may be more likely to drop out with a resultant over-estimation of the degree of consensus in final results. (12)

A single group email will be sent midway through the 21-day response period of each round as a reminder to participants to complete their questionnaires.

Pilot exercise

This Delphi exercise will be piloted by members of the steering committee who meet study eligibility criteria. The methodology may be revised subsequent to the pilot exercise. (28)

Protocol deviations

Deviations from the protocol during execution of the eDelphi process will be documented in the final report with justifications.

Ethics

Ethical approval for this work will be sought from the University of Galway's College of Medicine, Nursing & Health Sciences (CMNHS) Research Ethics Committee (REC). Potential participants will be fully informed about the background and nature of the study, their rights and expectations of them via an invitation email. The first questions in the survey will ask if the potential participant is over 18 years of age, if they have read and understood the participant information leaflet, if they are willing to consent to participate, and if yes, to proceed to the next question. If the answer to any of these questions is no, they will receive a message of thanks and may not proceed any further.

Participants have the right to withdraw from the survey at any time without the expectation of an explanation, but once responses have been submitted to either round of the survey, those responses cannot not be withdrawn from the analysis since data will have been pooled anonymously. This will be outlined in the information leaflet.

Contact details of participants will be retained by the study investigator only for the purpose of sending reminder emails to complete survey rounds if necessary.

The study will be conducted in accordance with GDPR requirements.

Discussion

This proposed Delphi exercise comprises one phase of a project that will determine if a clinical signs, symptoms and/or biomarkers tool to predict presence of biofilm in chronic diabetic foot ulcers (DFU) can be developed.

The Delphi technique has been adopted by many healthcare fields and is often modified to the degree that a uniform method is used by few researchers.^(15, 30, 31) This has been criticised as a threat to the validity and reliability of Delphi research findings and an editorial commentary from 2021 proposes that strict adherence to methodology could result in lower agreement between participants and more acceptance of Delphi findings.^(15, 32) Furthermore, a systematic review from 2017 reported that "Clear recommendations on conduct of Delphi studies and a reporting standard for their publication in peer-reviewed journals to date are not available."⁽³³⁾ Other authors also report issues with the Delphi process. Keeney et al. wrote that "there are no universally agreed criteria for the selection of experts, and no guidance exists on the minimum or maximum number of experts on a panel; rather it appears to be related to common sense and practical logistics."⁽³⁴⁾ Falzarano et al. state that "The literature does not suggest a set percent agreement; however, many studies use 80%."⁽³⁵⁾ Williamson et al. write that "The issue of the

impact of panel composition on Delphi performance has seldom been investigated in general.”
(12)

We convened a steering committee of nine internationally based wound care clinicians and researchers to apply collective knowledge and research experience to resolving Delphi methodological dilemmas and thus optimise the rigour of this project. The following is an illustration of our discussion and decision-making process for finalising the number and nature of panels to be included in our project.

In the context of core outcome sets, Williamson et al. ⁽¹²⁾ argue that one homogenous participant group will produce outcomes relevant to one stakeholder group only. If heterogeneity is introduced into that group, there is a risk that resulting outcomes will reflect the relative proportion of stakeholders in the panel or on weightings that may be used for different groups. They advise a multiple participant-group approach comprising distinct stakeholder groups when differing stakeholder opinions are expected. ⁽¹²⁾ Our committee had to choose between recruiting a single participant group of biomedical researchers, a single participant group of clinicians, a single participant group of biomedical researchers and clinicians or two separate clinical and biomedical research participant groups.

A series of arguments for each scenario were presented as follows: it was proposed that a single participant group of biomedical researchers would be familiar with the relevant literature and have a perspective that clinical personnel might not. The committee was then asked how immersion in relevant literature or research data might shape biomedical researcher perspective, for example, it is generally reported that biofilm in chronic wounds is difficult to visualise. ⁽⁵⁾ Finally, the committee was asked to consider the applicability of laboratory-based biofilm research knowledge involving *in vitro* and/or pre-clinical (animal models) to the day-to-day clinical management of wounds involving biofilms.

In terms of recruiting a clinical participant group, it was presented that the outcome of this research is intended exclusively for clinical personnel. In addition, clinicians are grounded in a clinical environment, but biofilm research is quite a niche area, and clinicians might not be up-to-date on relevant literature. Finally, given that the overall project hopes to minimise clinical error, the committee was asked whether clinical involvement at the Delphi phase would align more with this objective than would biomedical researcher involvement.

Regarding a single, mixed group of participating clinicians and biomedical researchers or two separate (by background) groups, it was proposed that both perspectives would be considered and that there would be potential for divergence between both due to differing perspectives.

Initially, the steering group split along a fault line of clinical vs. biomedical research perspectives and focused on whether a single clinical participant group or two separate clinical and biomedical research groups should be created.

Those in favour of a clinical group proposed that it can be difficult to apply academic/laboratory knowledge to a clinical context without relevant experience. Secondly, the research project of which this eDelphi exercise is a component pertains to clinical experience, and so the population should emphasise the clinical. Finally, the end product of this research, if successful, will exclusively serve clinical personnel. Conversely, it was argued that while two separate biomedical research and clinical groups may result in divergence of opinion, all opinions matter and should be captured to reduce the risk of important information being omitted. The debate then evolved into a discussion on how researchers should be defined for the purpose of this project and if perhaps, they should be seen as clinical personnel who engage in research. However, clinicians who engage in biomedical research are a minority in the wound care community and consequently there is a risk of having too small a sample size.

Ultimately it was agreed that we recruit a group of participants populated predominantly by clinicians with experience in treating wounds but that we also be open to wound care clinicians with interest and experience in research. The committee unanimously agreed that this was the best course of action to ensure that a participant group capable of meeting our research needs could be created.

Ambiguities within each of the eleven Delphi methodological areas reported by Williamson et al. ⁽¹²⁾ were discussed by the steering committee in a similar fashion and finalised into the methodology reported herein.

Conclusion

Internal validity of research findings is contingent on the rigour within the methodology giving rise to those findings. A rigorous methodology reduces the risk of introducing bias into research through systematic error. Concerns have been expressed in regard to uniformity of methodology across Delphi studies and the cost that this may bring in terms of reliability and validity of study findings. In light of these concerns, we finalised the process for this Delphi study through review and discussion of methodological dilemmas as well as experiential contributions from clinicians and researchers, believing this to be the most the most prudent and balanced approach, given the current state of the Delphi art.

We conclude as a group that the Delphi method would greatly benefit from in-depth review and quantitative examination in order to develop a more uniform, standardised and rigorous process.

Implications for clinical practice or future research

- When completed, the findings of this work will help conceptualise current clinical opinion on what does or does not constitute a clinical sign, symptom and/or biomarker of biofilm in chronic wounds.
- There is potential to review and research the Delphi process to develop a more standardised, rigorous and uniform methodology.

Conflict of interest

The authors declare no conflicts of interest.

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Author contributions

Conception and design: JDI, DS, PMC, CH, JVS, JPO, DG, GG

Data analysis and interpretation: JDI, DRS, JVS

Manuscript draft: JDI, GG

Critical revision of the manuscript: JDI, DS, PMC, DRS, CH, JVS, JPO, DG, GG

Final approval of the manuscript: JDI, DS, PMC, DRS, CH, JVS, JPO, DG, GG

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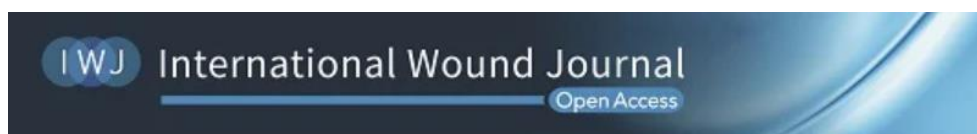
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3.4.3 *Electronic Delphi study results manuscript*

This subsection exhibits a report published in the open access ‘International Wound Journal’ (IWJ) that includes the results for this phase of the project (Ivory et al, 2025b).

Study materials including a draft invitation email and participation information leaflet, a draft survey questionnaire, draft reminder emails to complete rounds 1 and 2, an approval letter from the University of Galway Research Ethics Committee (REC) and an example of a feedback document provided to participants can be found in Appendix 2.



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REVIEW ARTICLE OPEN ACCESS

Clinical Signs and Symptoms of Biofilm in Chronic Wounds. What Do Practitioners Think? Consensus Through an Electronic Delphi Survey

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Key words: biofilms | chronic wounds | consensus | electronic Delphi | signs and symptoms

Abstract

This study aimed to gain clinician consensus on which signs/symptoms reported to be indicative of biofilm in chronic wounds are likely to be so. An international, two-round eDelphi process including wound care clinicians ran from December 2023-February 2024. Participants rated 26 items on a 9-point Likert scale. Consensus to include: $\geq 70\%$ of respondents rate an item 7-9, $\leq 15\%$ rate it 1-3. Consensus to exclude: $\geq 70\%$ of respondents rate an item 1-3, $\leq 15\%$ rate it 7-9. Eleven items (Visual indicators [a shiny, slimy, persistent layer, easily removed, returns quickly without frequent intervention]; failure to respond to antimicrobials; infection >30 days duration; poor quality granulation tissue; stalled wound despite optimal management; persistent/prolonged inflammation; wound >6 weeks duration; soft tissue deterioration despite antimicrobials/debridement; signs of local infection; tunnelling/undermining; presence of slough) achieved consensus to include status. To our knowledge, consensus work on this topic has not previously been performed on such a wide scale. When examined alongside similar work, clinical opinion on the matter lacks coherence. We hope that these findings will help

direct us toward greater cohesiveness. The work supports a need for research to quantify the predictive abilities of signs and symptoms reported to be indicative of biofilm in chronic wounds.

Summary

- In the absence of a readily available definitive test to predict biofilm presence in chronic wounds at the bedside, clinicians may look for clinical sign and symptom indicators instead.
- This electronic Delphi study aimed to gain consensus from an international panel of experienced wound care clinicians on which clinical signs and symptoms reported in the literature to be indicative of biofilm in chronic wounds were most likely to be so.
- From a list of 26 clinical signs and symptom items collated from the literature by means of an extensive scoping review, 11 met this study's 'consensus to include' criterion.
- When the findings of this research are viewed in the context of other related work on this topic, it becomes apparent that clinical opinion as yet lacks cohesiveness.

1 | Introduction

Microorganisms can present as a free living, planktonic phenotype or as sessile aggregates of cells known as a biofilm [1]. The biofilm phenotype occurs when bacterial cells begin to form attachments to a surface structure that are initially reversible but become more permanent and secure, and as they do, cells aggregate to each other and fixed microcolonies surrounded by a self-produced protective extracellular matrix start to develop [2, 3]. These colonies expand through growth and through recruitment of other bacterial cells to form mature, complex biofilms characterised by oxygen, nutrient and other substrate gradients which create a diverse microenvironment and physiological heterogeneity amongst the cells dwelling within [3, 4]. The biofilm phenotype predominates, and it forms and thrives in diverse environments including water systems, sediments and soil, as dental plaque, medical devices and living tissue [3-7].

Biofilms can be pathological and have been implicated in infections of various tissues of the body for example, lung infections in cystic fibrosis patients, osteomyelitis, otitis media, periodontitis, infective carditis and chronic wounds, as well as infections associated with medical devices such as catheters, pacemakers and prosthetic heart valves [8, 9]. The pathology of biofilm infections is persistent and progressive, largely arising out of the host's local

inflammatory response to biofilm presence and they tend to be chronic and difficult to treat [8]. Clinical features common to chronic biofilm infections include persistent infection of more than seven days' duration, signs of infection (tumour, rubor, dolor, loss of function, low-grade fever), failure of antibiotic treatment and recurrence of infection, and systemic signs and symptoms of infection that resolve with antibiotic therapy but recur on cessation of treatment [8].

In terms of chronic wounds, biofilms can be characterised as 'aggregate microorganisms that have unique characteristics and enhanced tolerance to treatment and the host defences. Wound biofilms are associated with impaired wound healing and signs and symptoms of chronic inflammation' [10]. The ability of biofilms to tolerate adverse environmental conditions significantly impairs the effectiveness of antimicrobials to manage wound bioburden, and according to Schultz et al. they are 'probably the most important single cause of persistent, delayed healing' after controlling for other factors such as venous or arterial insufficiency, pressure or underlying systemic diseases [7, 11]. Wolcott et al. wrote that in spite of having dissimilar aetiologies, chronic wounds have specific biochemical and cellular similarities; in essence, they are fixed in a persistent inflammatory state with elevated levels of pro-inflammatory cytokines, proteases and neutrophils and this state may be a result of interference from biofilm communities [12]. They proposed that these communities are difficult to eradicate and thus tend to perpetuate inflammation which promotes chronicity of these wounds.

Biofilms are prevalent in chronic wounds, estimated to be present in 60 – 78%, possibly over 90% of these injuries, yet they have only been found in 6% of acute wounds [13-15].

Biofilms can be difficult to diagnose in chronic wounds; standard culturing methods can underestimate the degree of bacterial colonisation and species diversity, and while advanced microscopy is effective, it is not feasible to incorporate as part of standard practice, meaning that clinicians may use clinical signs and symptoms to determine their presence in chronic wounds at the bedside [8, 16-18]. Furthermore, given the high prevalence estimates for biofilm in chronic wounds it is likely that a significant proportion of patients are seen outside the hospital setting in the community, where microscopic tests are even less feasible. While healthcare professionals may rely on specific signs and symptoms to confirm biofilm presence, little validation work has been done to confirm their predictive abilities [11, 19, 20]. Biofilm targeting wound management strategies incorporating debridement, therapeutic cleansing with surfactants and antiseptics, and antimicrobial wound dressings are recommended, and there are agents available that reportedly have antibiofilm capabilities, but supporting evidence is limited

[2, 10, 11, 15]. A critical concern arises then in terms of the effectiveness of antibiofilm strategies and interventions in that if we cannot easily determine biofilm presence in chronic wounds, how can we be sure that it was there in the first place, that our strategies have been effective and that its removal actually facilitated healing?

We completed a scoping review of the literature in which we collated a list of clinical signs or symptoms that were reported to be indicative of biofilm in chronic wounds [21].

The purpose of this study is to refine this list by gaining consensus from an international panel of experienced wound care clinical personnel through an electronic Delphi process on which of its items most likely indicate biofilm presence in chronic wounds.

2 | Material and Methods

The methodology for this anonymous, two-round Delphi survey was developed by an international steering committee comprising nine experienced wound care clinicians and researchers and is based on guidance for conduct of the Delphi technique provided by *The COMET handbook: version 1.0* [22]. A protocol has been published previously [23].

2.1 | Ethical Approval

Ethical approval was sought from and granted by the University of Galway Research Ethics Committee (REC) (application reference number: 2023.08.015). Informed consent was obtained from all participants.

2.2 | Recruitment, Sample Size and Eligibility Criteria

Attempts to contact 18 wound care organisations located worldwide were made with a request for assistance with this Delphi study. Gatekeepers of responding organisations were asked to distribute an invitation email that included a participant information leaflet and a link to the first round of the study to organisation members. The invitation email also included a snowballing element in which recipients were asked to consider forwarding it to colleagues in their field who were not necessarily organisation members.

The study aimed to recruit at least 30 clinical personnel currently active in wound care practice including nursing, podiatry and medicine to form a single participant panel. There is no power calculation for statistically determining sample sizes for Delphi work, and finalising this number is often based on matters of practicality [23]. The sample size for this work was decided

after discussion between steering committee members and centered around factors of attrition, estimate of external validity, representativeness and participant appropriateness and practicality.

Eligible participants could be located in any geographical region worldwide and had to possess three or more years clinical experience, 50% or greater of which must have been spent in a wound care environment. Response options for eligibility questions and the consent to participate question were a dichotomous *yes* or *no*. Choosing the *no* option for any eligibility question or for the consent to participate question eliminated the responder from the questionnaire at that point.

2.3 | Data Collection

QuestionPro survey software [24] was the platform of choice for creation and distribution of questionnaires.

2.3.1 | Round One

Consenting participants were asked to provide demographic details in terms of years of clinical experience, years of wound care experience, specialty (nursing, podiatry, medicine, other), field of practice (general, wound, ostomy, continence nursing, gerontology, dermatology, other), practice setting (private, community, nursing home, hospital inpatient or ambulatory, other) and country of practice.

A list of 26 clinical signs and symptoms (items) reported in the literature to be indicative of biofilm in chronic wounds was derived through a comprehensive scoping review (Table 1) [21]. The list was randomised in Microsoft Excel, and participants were asked to rate each item along a 9-point Likert scale of not likely to be indicative of biofilm in chronic wounds (1-3), somewhat likely to be indicative of biofilm in chronic wounds (4-6), and very likely to be indicative of biofilm in chronic wounds (7-9).

Table 1 | Signs and symptoms reported in the literature to be indicative of biofilm in chronic wounds collated through a scoping review of the literature [21].

Sign/symptom
Visual indicators: A shiny, slimy layer on a non-healing wound surface that persists despite autolytic or enzymatic debridement but can be removed easily with physical removal techniques such as cleansing or mechanical debridement. The surface reforms quickly (1-2 days) in the absence of frequent intervention, for example, cleansing, debridement.
Failure to respond as expected to antimicrobial therapies, for example, systemic antibiotics and/or topical antimicrobials/antiseptics. Infection may re-emerge when treatment is completed.
Wound fails to close or progress to healing despite optimal wound/patient management.
Increased exudate.
Presence of slough or fibrin.
Signs of local infection, for example, erythema, oedema, serous effusion, new areas of breakdown, pain etc.
Persistent or prolonged (low-level) inflammation.
Poor quality granulation tissue, for example, discoloured, friable, fragile or hypergranulation.
Signs of necrotic tissue/eschar.
Infection lasting more than 30 days or persistent cycles of recurrence (waxing and waning).
Favourable response to therapies such as corticosteroids, tumour necrosis factor (TNF) α inhibitors, or multimodal strategies such as physical debridement, cleansing and topical antimicrobial agents and dressings.

Malodour.
Negative cultures despite signs/suspicion of clinical infection.
Wound pain/pain at site.
Low-level/increased erythema.
Wound edge, for example, maceration, non-physiological colour changes of the wound margin, hyperkeratosis.
Swelling/wound bed with pale oedema.
Debris or foreign objects in the wound, for example, pins, mesh, prosthetic devices.
Soft tissue deterioration despite antibiotic treatment and/or surgical debridement. Tunnelling (along tissue structures, degradation along solid structures such as tendon or bone) and/or undermining (erosion of the subcutaneous layer under the wound edge).
Temperature. Sometimes low-grade fever.
History of systemic antibiotics in the last 12 months, prior or current use of antimicrobials.
Wound duration of more than 6 weeks.
Extreme tolerance to host immune defence.
Sepsis
Other patient history indicators, for example, change in the patient's overall wellbeing or quality of life.

Participants were also given the option to justify their rating score for each item. Finally, they were asked to provide an email address to receive a link to the second round of the questionnaire. A reply to this request was not mandatory

The first round was open for 21 days. One week after initiation, organisation gatekeepers were asked to distribute a single reminder to complete via email to invitation recipients.

2.3.2 | Definition of Consensus

A consensus decision to keep or reject an item on the list was made based on the following scoring system [25]:

- Consensus to include: $\geq 70\%$ of participants rate an item 7-9 and $\leq 15\%$ rate it 1-3.
- Consensus to exclude: $\geq 70\%$ of participants rate an item 1-3 and $\leq 15\%$ rate it 7-9.
- No consensus: Any item not meeting the criteria above.

Items receiving a *no consensus* score in round one were carried forward to round two of the process.

2.3.3 | Feedback Between Rounds

Item ratings were examined at the end of round one and a feedback pdf document for each item to be brought forward to round two (ie., items receiving a *no consensus* score [see section 2.3.2 *Definition of consensus*]) was created (Supporting information). Each document reported the number of responding and non-responding participants and the distribution of participant scores in terms of mean/standard deviation and median/interquartile range/1st and 3rd quartiles. In addition, a frequency distribution table, bar plot and boxplot illustrating how scores spread across the 9-point Likert scale and a list of score justifications provided by responding participants were included. Each document was then attached to its corresponding item in the round two questionnaire.

2.3.4 | Round Two

A questionnaire was distributed to participants who provided an email address at the end of round one. These participants were asked to provide demographic information (see section 2.3.1 *Round one*) and to rate a list of all items that received a *no consensus* score in round one along a 9-point Likert scale (see section 2.3.1 *Round one*). Items receiving a *consensus to include* score (see section 2.3.2 *Definition of consensus*) at the end of round two were retained. This round was kept open for 21 days, and a reminder to complete email was distributed one week after initiation of the round.

2.4 | Statistics

Data relating to demographics and participant scoring of sign and symptom items was presented in terms of number and percent of total respondents. Mean/standard deviation,

median/interquartile range and 95% confidence interval values were calculated for participant scoring of sign and symptom items.

3 | Results

One hundred and three clinicians consented to participate, and 56 participants completed round one (i.e., responded to all questions). Twenty-five of these initiated round two, and 23 completed it fully.

Twenty-two countries were represented in round one (Figure 1a). Twenty-three percent of the respondents came from the United Kingdom (UK) followed by 16.5% from Spain. Thirteen countries were represented in round two (Figure 1b). Forty percent of participants came from the UK and Spain.

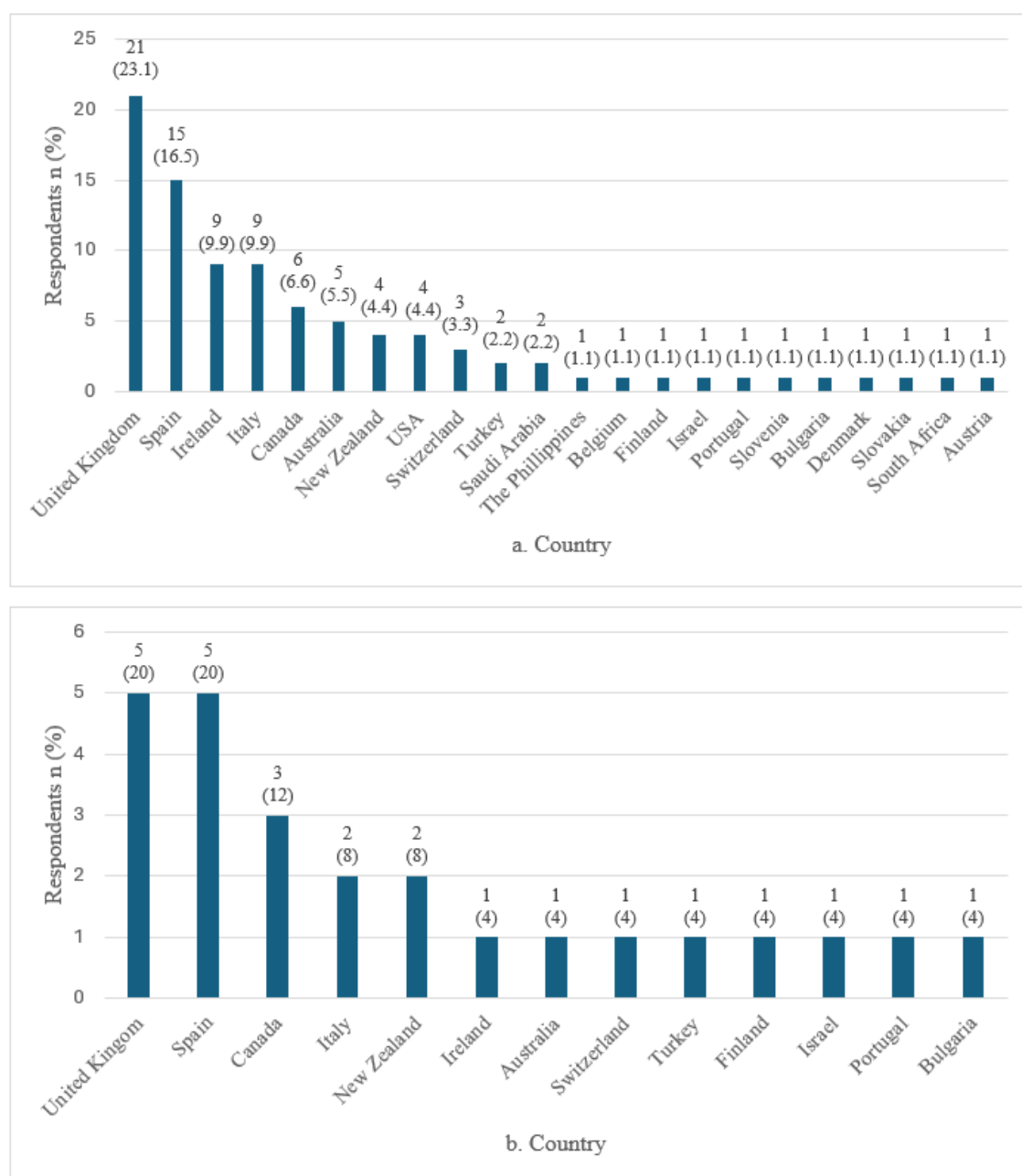


Figure 1 | Countries of origin of (a) round one (total number of respondents = 91, % missing values = 11.7) and (b) round two (total number of respondents = 25, % missing values = 75.73) participants.

In round one, the majority of responding participants (57.6%) had 21 years or more clinical experience, and 35% said that they had 21 years or more of experience in a wound care environment (Table 2.). The majority (84%) of round two participants also had 21 years or more clinical experience, and 44% reported having 21 years or more experience in wound care environment (Table 2.).

Table 2 | Clinical experience and wound care experience by number of years for round one and round two participants.

	Clinical experience n (%)		Wound care experience n (%)	
	Round 1	Round 2	Round 1	Round 2
1.5 – 3 years	0 (0)	0 (0)	8 (8.25)	0 (0)
3 – 5 years	9 (9.1)	0 (0)	8 (8.25)	0 (0)
6 – 10 years	6 (6.1)	0 (0)	11 (11.34)	0 (0)
11 – 15 years	14 (14.14)	3 (12)	15 (15.5)	6 (24)
16 – 21 years	13 (13.13)	1 (4)	21 (21.7)	8 (32)
21 + years	57 (57.6)	21 (84)	34 (35.1)	11 (44)
Total respondents	99 (96.12)	25 (24.3)	97 (94.2)	25 (24.3)
Missing values	4 (3.9)	78 (75.73)	6 (5.83)	78 (75.73)

A nursing background was reported by 75% of responding round one participants (Table 3), and 56% reported practicing in wound, ostomy and continence nursing (Table 4).

Table 3 | Specialty area of round one and round two participants.

	Round 1 n (%)	Round 2 n (%)
Nursing	74 (75.51)	15 (60)
Podiatry	7 (7.14)	3 (12)
Medicine	13 (13.3)	7 (28)
Other	4 (4.1)	0 (0)
Total respondents	98 (95.15)	25 (24.3)
Missing values	5 (4.9)	78 (75.73)

Table 4 | Field of practice of round one and round two participants.

	Round 1 n (%)	Round 2 n (%)
General practice	9 (9.4)	5 (20)
Wound, ostomy & continence nursing	54 (56.3)	9 (36)
Gerontology	2 (2.21)	1 (4)
Dermatology	3 (3.12)	4 (16)
Other	27 (28.12)	6 (24)
Prefer not to say	1 (1.04%)	
Total respondents	96 (93.2)	25 (24.3)
Missing values	7 (6.8)	78 (75.73)

Other fields of practice included community nursing, critical care, biomechanics in wound care, diabetic foot, plastic and reconstructive surgery (burns and hard-to-heal wounds), and orthopaedics. In terms of setting, 34% of round one participants practiced in a hospital inpatient setting (Table 5).

Table 5 | Practice setting of round 1 and round 2 participants.

	Round 1 n (%)	Round 2 n (%)
Private practice	16 (16.7)	2 (8)
Community	17 (17.71)	3 (12)
Nursing home	2 (2.1)	0 (0)
Hospital (inpatient)	33 (34.4)	9 (36)
Hospital (outpatient)	18 (18.8)	8 (32)
Other	10 (10.42)	3 (12)
Total respondents	96 (93.2)	25 (24.3)
Missing values	7 (6.8)	78 (75.3)

Other practice settings included joint primary/secondary care, university, and outpatient wound clinic with inpatient consultation plus regional telehealth settings. Most of the participants in

round two (60%) came from a nursing background (Table 3), and 36% worked in the field of wound, ostomy and continence nursing (Table 4). Other fields included orthopaedic/diabetic foot surgery, plastics and burns, general surgery, diabetic foot, and education/academia.

A hospital inpatient setting represented 36% of round two participants respectively (Table 5) and other settings included community and university.

As shown in tables 6 and 7, of the 26 items rated in round one, eight (visual indicators [a persistent shiny, slimy layer on the wound that is easily removed but returns quickly in the absence of frequent intervention]; failure to respond as expected to antimicrobial therapies; infection lasting more than 30 days/persistent cycles of recurrence [waxing and waning]; poor quality granulation tissue e.g., discoloured, friable, fragile or hypergranulation; wound failing to progress to healing despite optimal management; persistent/prolonged inflammation; wound duration of more than 6 weeks and soft tissue deterioration despite therapy) met the *consensus to include* criterion (see Table A1 for descriptive statistics). No items in this round were voted to meet the *consensus to exclude* criterion. The 18 remaining items were thus left with a status of *no consensus* and retained for voting in round two.

As per tables 6 and 7, of the 18 items with a *no consensus* status after round one, three items (signs of local infection; tunnelling and/or undermining and presence of slough or fibrin) met the *consensus to include* criterion in round two (see Table A1 for descriptive statistics). No items in this round were voted to meet the *consensus to exclude* criterion, and the remaining 15 items were therefore left with a status of *no consensus*.

Table 6 | Sign and symptom items that received a *consensus to include* status in either round one or round two.

A shiny, slimy layer on a non-healing wound surface that persists despite autolytic or enzymatic debridement but can be removed easily with physical removal techniques such as cleansing or mechanical debridement. The surface reforms quickly (1-2 days) in the absence of frequent intervention (for example, cleansing, debridement).
Failure to respond as expected to antimicrobial therapies (e.g., systemic antibiotics and/or topical antimicrobials/antiseptics). Infection may re-emerge when treatment is completed.

Infection lasting more than 30 days or persistent cycles of recurrence (waxing and waning).
Poor quality granulation tissue (e.g., discoloured, friable, fragile or hypergranulation).
Wound fails to close or progress to healing despite optimal wound/patient management.
Persistent or prolonged (low-level) inflammation.
Wound duration of more than 6 weeks.
Soft tissue deterioration despite antibiotic treatment and/or surgical debridement.
Signs of local infection (e.g. erythema, oedema, serous effusion, new areas of breakdown, pain, etc.)
Tunnelling (along tissue structures, degradation along solid structures such as tendon or bone) and/or undermining (erosion of the subcutaneous layer under the wound edge).
Presence of slough or fibrin.

Table 7 Rating scores along a 9-point Likert scale of 1 – 3 = <i>not likely to be indicative of biofilm in chronic wounds</i> , 4 – 6 = <i>somewhat likely to be indicative of biofilm in chronic wounds</i> and 7 – 9 = <i>very likely to be indicative of biofilm in chronic wounds</i> for all 26 signs and symptom items reported to be indicative of biofilm in chronic wounds.																				
Green shading represents a <i>consensus to include</i> status, and amber shading represents a <i>no consensus</i> status.																				
Clinical sign and symptom items	Round 1 rating scores % rating (n)	Consensus	Round 2 rating scores % rating (n)	Consensus	Final decision															
A shiny, slimy layer on a non-healing wound surface that persists despite autolytic or enzymatic debridement but can be removed easily with physical removal techniques such as cleansing or mechanical debridement. The surface reforms quickly (1-2 days) in the absence of frequent intervention (for example, cleansing, debridement).	<table><tr><td>1 - 3</td><td>4 - 6</td><td>7 - 9</td></tr><tr><td>4.62</td><td>4.62</td><td>90.8</td></tr><tr><td>(3)</td><td>(3)</td><td>(59)</td></tr></table>	1 - 3	4 - 6	7 - 9	4.62	4.62	90.8	(3)	(3)	(59)	Include	<table><tr><td>1 - 3</td><td>4 - 6</td><td>7 - 9</td></tr><tr><td>-</td><td>-</td><td>-</td></tr></table>	1 - 3	4 - 6	7 - 9	-	-	-	N/A	Include
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Failure to respond as expected to antimicrobial therapies (for example, systemic antibiotics and/or topical antimicrobials/antiseptics). Infection may re-emerge when treatment is completed.	<table><tr><td>1 - 3</td><td>4 - 6</td><td>7 - 9</td></tr><tr><td>1.72</td><td>12.1</td><td>86.21</td></tr><tr><td>(1)</td><td>(7)</td><td>(50)</td></tr></table>	1 - 3	4 - 6	7 - 9	1.72	12.1	86.21	(1)	(7)	(50)	Include	<table><tr><td>1 - 3</td><td>4 - 6</td><td>7 - 9</td></tr><tr><td>-</td><td>-</td><td>-</td></tr></table>	1 - 3	4 - 6	7 - 9	-	-	-	N/A	Include
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Infection lasting more than 30 days or persistent cycles of recurrence (waxing and waning).	<table><tr><td>1 - 3</td><td>4 - 6</td><td>7 - 9</td></tr><tr><td>3.5 (2)</td><td>12.07 (7)</td><td>84.5 (2)</td></tr></table>	1 - 3	4 - 6	7 - 9	3.5 (2)	12.07 (7)	84.5 (2)	Include	<table><tr><td>1 - 3</td><td>4 - 6</td><td>7 - 9</td></tr><tr><td>-</td><td>-</td><td>-</td></tr></table>	1 - 3	4 - 6	7 - 9	-	-	-	N/A	Include
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3.5 (2)	12.07 (7)	84.5 (2)															
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Poor quality granulation tissue (for example, discoloured, friable, fragile or hypergranulation).	<table><tr><td>1 - 3</td><td>4 - 6</td><td>7 - 9</td></tr><tr><td>6.7 (5)</td><td>13.33 (10)</td><td>80 (60)</td></tr></table>	1 - 3	4 - 6	7 - 9	6.7 (5)	13.33 (10)	80 (60)	Include	<table><tr><td>1 - 3</td><td>4 - 6</td><td>7 - 9</td></tr><tr><td>-</td><td>-</td><td>-</td></tr></table>	1 - 3	4 - 6	7 - 9	-	-	-	N/A	Include
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Wound fails to close or progress to healing despite optimal wound/patient management.	<table><tr><td>1 - 3</td><td>4 - 6</td><td>7 - 9</td></tr><tr><td>5.2 (3)</td><td>19 (11)</td><td>75.9 (44)</td></tr></table>	1 - 3	4 - 6	7 - 9	5.2 (3)	19 (11)	75.9 (44)	Include	<table><tr><td>1 - 3</td><td>4 - 6</td><td>7 - 9</td></tr><tr><td>-</td><td>-</td><td>-</td></tr></table>	1 - 3	4 - 6	7 - 9	-	-	-	N/A	Include
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Persistent or prolonged (low-level) inflammation.	<table><tr><td>1 - 3</td><td>4 - 6</td><td>7 - 9</td></tr><tr><td>1.4</td><td>23.61</td><td>75.01</td></tr><tr><td>(1)</td><td>(17)</td><td>(54)</td></tr></table>	1 - 3	4 - 6	7 - 9	1.4	23.61	75.01	(1)	(17)	(54)	Include	<table><tr><td>1 - 3</td><td>4 - 6</td><td>7 - 9</td></tr><tr><td>-</td><td>-</td><td>-</td></tr></table>	1 - 3	4 - 6	7 - 9	-	-	-	N/A	Include			
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(1)	(17)	(54)																					
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Wound duration of more than 6 weeks.	<table><tr><td>1 - 3</td><td>4 - 6</td><td>7 - 9</td></tr><tr><td>6.6</td><td>19.7</td><td>73.8</td></tr><tr><td>(4)</td><td>(12)</td><td>(45)</td></tr></table>	1 - 3	4 - 6	7 - 9	6.6	19.7	73.8	(4)	(12)	(45)	Include	<table><tr><td>1 - 3</td><td>4 - 6</td><td>7 - 9</td></tr><tr><td>-</td><td>-</td><td>-</td></tr></table>	1 - 3	4 - 6	7 - 9	-	-	-	N/A	Include			
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(4)	(12)	(45)																					
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Soft tissue deterioration despite antibiotic treatment and/or surgical debridement	<table><tr><td>1 - 3</td><td>4 - 6</td><td>7 - 9</td></tr><tr><td>5.3</td><td>24.6</td><td>70.2</td></tr><tr><td>(3)</td><td>(14)</td><td>(40)</td></tr></table>	1 - 3	4 - 6	7 - 9	5.3	24.6	70.2	(3)	(14)	(40)	Include	<table><tr><td>1 - 3</td><td>4 - 6</td><td>7 - 9</td></tr><tr><td>-</td><td>-</td><td>-</td></tr></table>	1 - 3	4 - 6	7 - 9	-	-	-	N/A	Include			
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5.3	24.6	70.2																					
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Signs of local infection (for example, erythema, oedema, serous effusion, new areas of breakdown, pain, etc.)	<table><tr><td>1 - 3</td><td>4 - 6</td><td>7 - 9</td></tr><tr><td>13.33</td><td>22.7</td><td>64</td></tr><tr><td>(10)</td><td>(17)</td><td>(48)</td></tr></table>	1 - 3	4 - 6	7 - 9	13.33	22.7	64	(10)	(17)	(48)	No consensus	<table><tr><td>1 - 3</td><td>4 - 6</td><td>7 - 9</td></tr><tr><td>8.33</td><td>12.5</td><td>79.2</td></tr><tr><td>(2)</td><td>(3)</td><td>(19)</td></tr></table>	1 - 3	4 - 6	7 - 9	8.33	12.5	79.2	(2)	(3)	(19)	Include	Include
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1 - 3	4 - 6	7 - 9																					
8.33	12.5	79.2																					
(2)	(3)	(19)																					

Tunnelling (along tissue structures, degradation along solid structures such as tendon or bone) and/or undermining (erosion of the subcutaneous layer under the wound edge).	<table><tr><th>1 - 3</th><th>4 - 6</th><th>7 - 9</th></tr><tr><td>13.23 (9)</td><td>32.4 (22)</td><td>54.42 (37)</td></tr></table>	1 - 3	4 - 6	7 - 9	13.23 (9)	32.4 (22)	54.42 (37)	No consensus	<table><tr><th>1 - 3</th><th>4 - 6</th><th>7 - 9</th></tr><tr><td>4.12 (1)</td><td>20.83 (5)</td><td>75 (18)</td></tr></table>	1 - 3	4 - 6	7 - 9	4.12 (1)	20.83 (5)	75 (18)	Include	Include
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13.23 (9)	32.4 (22)	54.42 (37)															
1 - 3	4 - 6	7 - 9															
4.12 (1)	20.83 (5)	75 (18)															
Presence of slough or fibrin.	<table><tr><th>1 - 3</th><th>4 - 6</th><th>7 - 9</th></tr><tr><td>13.9 (9)</td><td>24.61 (16)</td><td>61.54 (40)</td></tr></table>	1 - 3	4 - 6	7 - 9	13.9 (9)	24.61 (16)	61.54 (40)	No consensus	<table><tr><th>1 - 3</th><th>4 - 6</th><th>7 - 9</th></tr><tr><td>0 (0)</td><td>29.2 (7)</td><td>70.83 (17)</td></tr></table>	1 - 3	4 - 6	7 - 9	0 (0)	29.2 (7)	70.83 (17)	Include	Include
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13.9 (9)	24.61 (16)	61.54 (40)															
1 - 3	4 - 6	7 - 9															
0 (0)	29.2 (7)	70.83 (17)															
Favourable response to therapies such as corticosteroids, TNF α inhibitors or multimodal strategies such as physical debridement, cleansing and topical antimicrobial agents and dressings.	<table><tr><th>1 - 3</th><th>4 - 6</th><th>7 - 9</th></tr><tr><td>11.9 (7)</td><td>23.72 (14)</td><td>64.41 (38)</td></tr></table>	1 - 3	4 - 6	7 - 9	11.9 (7)	23.72 (14)	64.41 (38)	No consensus	<table><tr><th>1 - 3</th><th>4 - 6</th><th>7 - 9</th></tr><tr><td>12.5 (3)</td><td>25 (6)</td><td>62.5 (15)</td></tr></table>	1 - 3	4 - 6	7 - 9	12.5 (3)	25 (6)	62.5 (15)	No consensus	No consensus
	1 - 3	4 - 6	7 - 9														
11.9 (7)	23.72 (14)	64.41 (38)															
1 - 3	4 - 6	7 - 9															
12.5 (3)	25 (6)	62.5 (15)															
Extreme tolerance to host immune defence.	<table><tr><th>1 - 3</th><th>4 - 6</th><th>7 - 9</th></tr><tr><td>8.62 (5)</td><td>31.03 (18)</td><td>60.34 (35)</td></tr></table>	1 - 3	4 - 6	7 - 9	8.62 (5)	31.03 (18)	60.34 (35)	No consensus	<table><tr><th>1 - 3</th><th>4 - 6</th><th>7 - 9</th></tr><tr><td>13.04 (3)</td><td>26.1 (6)</td><td>60.9 (14)</td></tr></table>	1 - 3	4 - 6	7 - 9	13.04 (3)	26.1 (6)	60.9 (14)	No consensus	No consensus
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8.62 (5)	31.03 (18)	60.34 (35)															
1 - 3	4 - 6	7 - 9															
13.04 (3)	26.1 (6)	60.9 (14)															

Increased exudate.	<table><tr><td>1 - 3</td><td>4 - 6</td><td>7 - 9</td></tr><tr><td>17.34 (13)</td><td>25.34 (19)</td><td>57.34 (43)</td></tr></table>	1 - 3	4 - 6	7 - 9	17.34 (13)	25.34 (19)	57.34 (43)	No consensus	<table><tr><td>1 - 3</td><td>4 - 6</td><td>7 - 9</td></tr><tr><td>4.2 (1)</td><td>41.2 (10)</td><td>54.2 (13)</td></tr></table>	1 - 3	4 - 6	7 - 9	4.2 (1)	41.2 (10)	54.2 (13)	No consensus	No consensus
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17.34 (13)	25.34 (19)	57.34 (43)															
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4.2 (1)	41.2 (10)	54.2 (13)															
Malodour	<table><tr><td>1 - 3</td><td>4 - 6</td><td>7 - 9</td></tr><tr><td>22.01 (14)</td><td>24.3 (17)</td><td>55.72 (39)</td></tr></table>	1 - 3	4 - 6	7 - 9	22.01 (14)	24.3 (17)	55.72 (39)	No consensus	<table><tr><td>1 - 3</td><td>4 - 6</td><td>7 - 9</td></tr><tr><td>8.33 (2)</td><td>50 (12)</td><td>41.2 (10)</td></tr></table>	1 - 3	4 - 6	7 - 9	8.33 (2)	50 (12)	41.2 (10)	No consensus	No consensus
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22.01 (14)	24.3 (17)	55.72 (39)															
1 - 3	4 - 6	7 - 9															
8.33 (2)	50 (12)	41.2 (10)															
Negative cultures despite signs/suspicion of clinical infection.	<table><tr><td>1 - 3</td><td>4 - 6</td><td>7 - 9</td></tr><tr><td>19.7 (12)</td><td>26.23 (16)</td><td>54.1 (33)</td></tr></table>	1 - 3	4 - 6	7 - 9	19.7 (12)	26.23 (16)	54.1 (33)	No consensus	<table><tr><td>1 - 3</td><td>4 - 6</td><td>7 - 9</td></tr><tr><td>8.33 (2)</td><td>25 (6)</td><td>66.2 (16)</td></tr></table>	1 - 3	4 - 6	7 - 9	8.33 (2)	25 (6)	66.2 (16)	No consensus	No consensus
	1 - 3	4 - 6	7 - 9														
19.7 (12)	26.23 (16)	54.1 (33)															
1 - 3	4 - 6	7 - 9															
8.33 (2)	25 (6)	66.2 (16)															
Debris or foreign objects in the wound (for example, pins, mesh, prosthetic devices).	<table><tr><td>1 - 3</td><td>4 - 6</td><td>7 - 9</td></tr><tr><td>24.15 (21)</td><td>21.84 (19)</td><td>54.02 (47)</td></tr></table>	1 - 3	4 - 6	7 - 9	24.15 (21)	21.84 (19)	54.02 (47)	No consensus	<table><tr><td>1 - 3</td><td>4 - 6</td><td>7 - 9</td></tr><tr><td>20 (5)</td><td>20 (5)</td><td>60 (15)</td></tr></table>	1 - 3	4 - 6	7 - 9	20 (5)	20 (5)	60 (15)	No consensus	No consensus
	1 - 3	4 - 6	7 - 9														
24.15 (21)	21.84 (19)	54.02 (47)															
1 - 3	4 - 6	7 - 9															
20 (5)	20 (5)	60 (15)															

History of systemic antibiotics in the last 12 months, prior or current use of antimicrobials.	<table><tr><td>1 - 3</td><td>4 - 6</td><td>7 - 9</td></tr><tr><td>20.7 (12)</td><td>29.3 (17)</td><td>50 (29)</td></tr></table>	1 - 3	4 - 6	7 - 9	20.7 (12)	29.3 (17)	50 (29)	No consensus	<table><tr><td>1 - 3</td><td>4 - 6</td><td>7 - 9</td></tr><tr><td>26.1 (6)</td><td>30.43 (7)</td><td>43.5 (10)</td></tr></table>	1 - 3	4 - 6	7 - 9	26.1 (6)	30.43 (7)	43.5 (10)	No consensus	No consensus
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20.7 (12)	29.3 (17)	50 (29)															
1 - 3	4 - 6	7 - 9															
26.1 (6)	30.43 (7)	43.5 (10)															
Sepsis	<table><tr><td>1 - 3</td><td>4 - 6</td><td>7 - 9</td></tr><tr><td>29.9 (20)</td><td>20.9 (14)</td><td>49.3 (33)</td></tr></table>	1 - 3	4 - 6	7 - 9	29.9 (20)	20.9 (14)	49.3 (33)	No consensus	<table><tr><td>1 - 3</td><td>4 - 6</td><td>7 - 9</td></tr><tr><td>16.7 (4)</td><td>25 (6)</td><td>58.33 (14)</td></tr></table>	1 - 3	4 - 6	7 - 9	16.7 (4)	25 (6)	58.33 (14)	No consensus	No consensus
	1 - 3	4 - 6	7 - 9														
29.9 (20)	20.9 (14)	49.3 (33)															
1 - 3	4 - 6	7 - 9															
16.7 (4)	25 (6)	58.33 (14)															
Swelling/wound bed with pale oedema.	<table><tr><td>1 - 3</td><td>4 - 6</td><td>7 - 9</td></tr><tr><td>21.43 (15)</td><td>34.3 (24)</td><td>44.3 (31)</td></tr></table>	1 - 3	4 - 6	7 - 9	21.43 (15)	34.3 (24)	44.3 (31)	No consensus	<table><tr><td>1 - 3</td><td>4 - 6</td><td>7 - 9</td></tr><tr><td>12.5 (3)</td><td>37.5 (9)</td><td>50 (12)</td></tr></table>	1 - 3	4 - 6	7 - 9	12.5 (3)	37.5 (9)	50 (12)	No consensus	No consensus
	1 - 3	4 - 6	7 - 9														
21.43 (15)	34.3 (24)	44.3 (31)															
1 - 3	4 - 6	7 - 9															
12.5 (3)	37.5 (9)	50 (12)															
Wound edge (for example, maceration, non-physiological colour changes of the wound margin, hyperkeratosis).	<table><tr><td>1 - 3</td><td>4 - 6</td><td>7 - 9</td></tr><tr><td>32.82 (21)</td><td>28.13 (18)</td><td>39.1 (25)</td></tr></table>	1 - 3	4 - 6	7 - 9	32.82 (21)	28.13 (18)	39.1 (25)	No consensus	<table><tr><td>1 - 3</td><td>4 - 6</td><td>7 - 9</td></tr><tr><td>25 (6)</td><td>25 (6)</td><td>50 (12)</td></tr></table>	1 - 3	4 - 6	7 - 9	25 (6)	25 (6)	50 (12)	No consensus	No consensus
	1 - 3	4 - 6	7 - 9														
32.82 (21)	28.13 (18)	39.1 (25)															
1 - 3	4 - 6	7 - 9															
25 (6)	25 (6)	50 (12)															

Signs of necrotic tissue/eschar.	<table><tr><td>1 - 3</td><td>4 - 6</td><td>7 - 9</td></tr><tr><td>32.4 (23)</td><td>29.6 (21)</td><td>38.03 (27)</td></tr></table>	1 - 3	4 - 6	7 - 9	32.4 (23)	29.6 (21)	38.03 (27)	No consensus	<table><tr><td>1 - 3</td><td>4 - 6</td><td>7 - 9</td></tr><tr><td>33.33 (8)</td><td>29.2 (7)</td><td>37.5 (9)</td></tr></table>	1 - 3	4 - 6	7 - 9	33.33 (8)	29.2 (7)	37.5 (9)	No consensus	No consensus
	1 - 3	4 - 6	7 - 9														
32.4 (23)	29.6 (21)	38.03 (27)															
1 - 3	4 - 6	7 - 9															
33.33 (8)	29.2 (7)	37.5 (9)															
Temperature. Sometimes low-grade fever.	<table><tr><td>1 - 3</td><td>4 - 6</td><td>7 - 9</td></tr><tr><td>35.4 (23)</td><td>29.23 (19)</td><td>35.4 (23)</td></tr></table>	1 - 3	4 - 6	7 - 9	35.4 (23)	29.23 (19)	35.4 (23)	No consensus	<table><tr><td>1 - 3</td><td>4 - 6</td><td>7 - 9</td></tr><tr><td>16.2 (4)</td><td>62.5 (15)</td><td>20.83 (5)</td></tr></table>	1 - 3	4 - 6	7 - 9	16.2 (4)	62.5 (15)	20.83 (5)	No consensus	No consensus
	1 - 3	4 - 6	7 - 9														
35.4 (23)	29.23 (19)	35.4 (23)															
1 - 3	4 - 6	7 - 9															
16.2 (4)	62.5 (15)	20.83 (5)															
Wound pain/pain at site.	<table><tr><td>1 - 3</td><td>4 - 6</td><td>7 - 9</td></tr><tr><td>21.74 (15)</td><td>44.92 (31)</td><td>33.34 (23)</td></tr></table>	1 - 3	4 - 6	7 - 9	21.74 (15)	44.92 (31)	33.34 (23)	No consensus	<table><tr><td>1 - 3</td><td>4 - 6</td><td>7 - 9</td></tr><tr><td>4.2 (1)</td><td>58.33 (14)</td><td>37.5 (9)</td></tr></table>	1 - 3	4 - 6	7 - 9	4.2 (1)	58.33 (14)	37.5 (9)	No consensus	No consensus
	1 - 3	4 - 6	7 - 9														
21.74 (15)	44.92 (31)	33.34 (23)															
1 - 3	4 - 6	7 - 9															
4.2 (1)	58.33 (14)	37.5 (9)															
Other patient history indicators (for example, change in the patient's overall wellbeing or quality of life).	<table><tr><td>1 - 3</td><td>4 - 6</td><td>7 - 9</td></tr><tr><td>36.9 (21)</td><td>35.1 (20)</td><td>28.08 (16)</td></tr></table>	1 - 3	4 - 6	7 - 9	36.9 (21)	35.1 (20)	28.08 (16)	No consensus	<table><tr><td>1 - 3</td><td>4 - 6</td><td>7 - 9</td></tr><tr><td>34.8 (8)</td><td>30.43 (7)</td><td>34.8 (8)</td></tr></table>	1 - 3	4 - 6	7 - 9	34.8 (8)	30.43 (7)	34.8 (8)	No consensus	No consensus
	1 - 3	4 - 6	7 - 9														
36.9 (21)	35.1 (20)	28.08 (16)															
1 - 3	4 - 6	7 - 9															
34.8 (8)	30.43 (7)	34.8 (8)															

Low-level/increased erythema.	No consensus			No consensus			No consensus		
	1 - 3	4 - 6	7 - 9	1 - 3	4 - 6	7 - 9	1 - 3	4 - 6	7 - 9
	38.5 (30)	34.62 (27)	26.93 (21)	20.83 (5)	37.5 (9)	41.7 (10)			
Consensus to include: $\geq 70\%$ of participants rate an item 7-9 and $\leq 15\%$ rate it 1-3. Consensus to exclude: $\geq 70\%$ of participants rate an item 1-3 and $\leq 15\%$ rate it 7-9. No consensus: Any item not meeting the criteria above. The first eight items received <i>consensus to include</i> status in round 1 and the three items subsequent received it in round 2.									

4 | Discussion

The purpose of this study was to gain consensus by means of an electronic Delphi study from an international panel of experienced wound care professionals on which clinical signs reported in the literature to be indicative of biofilm in chronic wounds are most likely to be so, and we found that from a list of 26 clinical sign and symptom items, 11 met our *consensus to include* criterion and 15 were left with a status of *no consensus* after two rounds of voting.

On comparing the items that met the *consensus to include* criterion to those that received a *no consensus* status, it is difficult to discern patterns that might point toward why our participants came to the observed conclusions. The option to justify voting decisions was provided in round 1 but uncovering potential patterns in this data would require a qualitative analysis which was not specified in the original study protocol [23]. However, in the next four paragraphs, we will present our own thoughts on why panel members might have voted the way they did.

It is interesting that the visual indicators (a persistent shiny, slimy layer on the wound that is easily removed but returns quickly in the absence of frequent intervention) item received the highest percentage of votes to award it *consensus to include* status of all items presented to participants in our study, notwithstanding that there is associated controversy. There are those who advocate the idea that biofilm might be confirmable in chronic wounds via visual indicators and early-stage supporting evidence has been presented [19, 26]. However, there are also those who are critical, saying that biofilms are difficult to see with the naked eye, that other features in the wound such as exudate, slough and debris may be erroneously conflated with biofilm presence, and that the visualisation approach is subjective, unproven and without basis and objective criteria [11, 27]. Biofilm infections are a complicated subject and are difficult to diagnose and treat [8, 9]. However, the simplicity principle posits that we are biased toward less complex explanations of phenomena that we encounter [28]. If this principle holds true, then it might make the idea of condensing the biofilm/wound dynamic into an easily explained, observed, and managed phenomenon such as a visible and removable layer on a wound surface that can be kept at bay with regular cleansing and debridement understandable and relatable. This bias toward simplicity may at least partially explain why the panel advocated so strongly to grant this item *consensus to include* status. The presence of slough or fibrin item also met the *consensus to include* criterion despite the cautions outlined above.

Six of the items meeting the *consensus to include* criterion are associated with infection, wound chronicity and failure to heal (failure to respond as expected to antimicrobial therapies; infection lasting more than 30 days/persistent cycles of recurrence [waxing and waning];

wound failing to progress to healing despite optimal management; wound duration of more than six weeks; soft tissue deterioration despite therapy and signs of local infection). It is consistently reported in the literature that biofilm is associated with delayed healing, and this might explain why those items were voted to meet this criterion.

Of the 15 items with *no consensus* status, 6 or 40% (increased exudate; malodour; swelling/wound bed with pale oedema; temperature; wound pain/pain at site; and low-level/increased erythema) constitute individual signs of local infection versus 3 or 27% (poor quality granulation tissue for example, discoloured, friable, fragile or hypergranulation; tunnelling/undermining and prolonged/persistent inflammation) of the 11 items meeting the *consensus to include* criterion [10]. One of the *consensus to include* items however refers to *signs* of infection. It could be that signs of infection are better conceptualized as a complete entity, rather than as individual components. Indeed, a validation study of clinical signs to assess bacterial burden in chronic wounds from 2009 concluded that ‘no one clinical sign may be sufficient to help clinicians decide whether a topical or systemic antibiotic should be prescribed’ [29]. The same study found that combining any three signs led to an increase in sensitivity.

It is not surprising that some items from the original list of 26 were left with a status of *no consensus*. Sepsis, defined as ‘life-threatening organ dysfunction caused by a dysregulated host response to infection’ [30], is an extreme and acute complication of infection involving multiple systems in the body. It is probably more surprising that this item was not completely excluded during the consensus process. Other items (other patient history indicators [change in the patient’s overall wellbeing or quality of life]; extreme tolerance to host immune defence and history of systemic antibiotics in the last 12 months/prior or current use of antimicrobials) could be seen as vague and/or all-encompassing in terms of the patient, and thus one might not intuitively associate them with a local wound context.

Placing our findings in the context of other similar research, an online survey by Metcalf and Bowler [31, 32] asked 81 wound care nurse practitioners from the United States and Europe how they knew if a wound contains biofilm. Of the 43 clinicians who responded to that question 20 (46.5%) reported that it is sometimes visible while 8 (18.6%) said that ‘it is not possible to see biofilm sometimes’ or ‘suggested indirect indicators of wound biofilm that is otherwise microscopic’. Ninety percent of our responding participants voted visual indicators as *very likely indicative of biofilm in chronic wounds* versus 4.6% who voted them *not likely to be indicative of biofilm in chronic wounds*. When asked about the impact of biofilm on wound

healing, 13 of 37 respondents (35%) in Metcalf and Bowler's study referred to antimicrobial tolerance whereas 86% of our responding participants voted the failure to respond as expected to antimicrobial therapies item as *very likely to be indicative of biofilm in chronic wounds* (1.7% voted this item as *not likely to be indicative of biofilm in chronic wounds*). 'Poor responses to standard care, including antimicrobial dressings or antibiotics.' ranked highest on a frequency chart of indirect signs that included among others, 'delayed healing', 'exudate', 'odour', and 'inflammation' in Metcalf and Bowler's work. Similarly, the wound failing to progress to healing despite optimal management item was voted *very likely to be indicative of biofilm in chronic wounds* in our study.

In a Delphi study by Haesler et al. which also queried clinical signs as indicators for biofilm [33], 14 international infection experts returned median (30 – 70% inter percentile range [IPR]) scores of 3.5 (1.7 - 5.6) and 2.0 (1.4 – 2.0) for rounds one and two of the process respectively that a 'surface substance on the wound that can be visualised is an indicator of potential biofilm in a wound' meaning that this group was opposed to the idea of visual signs as indicators of biofilm in chronic wounds. The views of participants in our Delphi work and those in the work of Haesler et al. thus ran contrary for this item. The participant populations in each study were different in terms of size, but more importantly, they may have been different in terms of composition. Haesler et al. recruited their participants from an international group that specialises in wound infection whereas our study expressed no such requirement and included participants from nursing, medicine and podiatry. While the majority of the participants in our study were highly experienced in terms of wound care, it is not inconceivable that a small group of clinical and research personnel chosen from a group that specialises in wound infection management might possess greater familiarity with the thinking and literature in that area and thus be more critical. On the other hand, of the remaining 10 clinical indicators in our study voted *very likely to be indicative of biofilm in chronic wounds*, five (failure to respond as expected to antimicrobial therapies; prolonged/persistent inflammation; signs of local infection; poor quality granulation tissue e.g., discoloured, friable, fragile or hypergranulation; and wound failing to progress to healing despite optimal management) corresponded to items receiving a vote of 'agreement' in the list of 11 clinical indicators presented to panel members in the Haesler et al. study. Four items in that study referenced antimicrobial failure (i.e., failure, unresponsiveness, recalcitrance and recurrence of delayed healing on cessation) versus a single general reference to same in our work. Increased exudate and low level erythema which received *agreement* status in the Haesler et al. study maintained a *no consensus* status after two rounds of voting in our study and were thus excluded from our final list. Five items voted *very*

likely to be indicative of biofilm in chronic wounds in our study (Infection lasting more than 30 days or persistent cycles of recurrence [waxing and waning]; wound duration of more than six weeks; soft tissue deterioration despite therapy; tunnelling and/or undermining and presence of slough or fibrin) were not presented to participants in the Haesler et al. study. In summary, regarding those clinical signs thought to point to biofilm in chronic wounds subjected to rounds of voting in both studies, there appeared to be more agreement between the potentially diverse participant groups than disagreement.

This carefully designed study brought together a large, multinational group of clinical personnel, greatly experienced in hands-on care of chronic wounds. They provided their opinion according to a Likert rating system on which items on list of all known signs and symptoms reported to point toward biofilm in chronic wounds are most likely to do so. To our knowledge this is the first time a consensus study on this topic of interest has been performed on such a wide scale. We anticipate that its findings might help direct us towards a greater degree of cohesiveness on a matter that can yet be seen as somewhat inconclusive.

4.1 | Strengths and Limitations of This Study

The Delphi technique is not flawless, and critique has been directed toward aspects such as methodological uniformity for example, with modifications often made to the process by researchers, uncertainty and lack of clear guidance in terms of its conduct, and need for a reporting standard for publication [22, 23, 34-37]. In addition, logistics and common sense would appear to drive selection of expert panellists rather than universally agreed criteria, and there is no clearly defined set percent agreement [23, 38, 39]. A significant strength of this study was the steering committee that convened to develop the study protocol [23]. This committee consisted of nine individuals from three different countries with considerable experience in wound care practice and research and also experience in Delphi methodology. The committee had use of a framework in the form of a comprehensive guidance document [22] which helped ensure that the development process was thorough and carefully thought through.

One hundred and three clinicians consented to participate in this Delphi process, 56 completed round 1 and of these, 25 responded to round two with 23 participants completing it fully. The completion rates therefore were 54% for round one and 92% for round two. The attrition rate was high; however, sample sizes ranging from 12 to > 300 have been reported across Delphi studies [22, 23] and the number of completers for each round of our study fall within that range. The guidance we used to direct our methodology [22] deems a response rate of 80% to be

satisfactory in most situations although they also report that there is no guidance on what an acceptable response rate actually is. Nevertheless, we aimed to recruit at least 30 participants and at the end of round 2 we had 23 which accounts for 76.7% of our anticipated sample size. The participant group in this study was strong. We hoped to recruit a global sample of wound care clinicians, and we were not disappointed, with representatives from 20 countries taking part. More than three specialties were represented, and nursing, a discipline very much found on the front line in terms of hands-on wound assessment and management, formed the majority. In addition, our participant group as a whole was greatly experienced, having for the most part between 11 and 21+ years of experience in the field.

4.2 | Protocol Deviations

We planned to retain items with a *no consensus* status at the end of round two and include them in a list of potential signs and symptoms of biofilm in chronic wounds to be subjected to testing against clinical samples. However, 15 items were left with this status, and we considered this to be too large and cumbersome a number to be practical in a clinical scenario.

We wanted to experiment with a Bland Altman analysis approach in an attempt to assess change in participant's scoring on items between rounds to examine the degree of consensus [22, 23, 40]. Ultimately, this was not possible because only items with 'no consensus' status passed through both rounds of voting, there was attrition between rounds, and mostly because the survey platform assigned a different response identification number to participants for each questionnaire round, making it impossible to match respondents to their responses across rounds. The platform records the IP address of each participant, but we could only match IP addresses across both rounds in the case of five respondents.

5 | Conclusion

This electronic Delphi exercise found that in the opinion of an international panel of experienced wound care clinicians there are 11 signs and symptoms that are very likely to indicate presence of biofilm in chronic wounds. Experienced clinical personnel are doubtless cognisant of the concept of biofilm in chronic wounds, of the risks it can pose and of the treatment strategies available, but when the findings of this study are viewed in unison with other opinion work on this topic it becomes apparent that wound care practitioners are not entirely coherent with regard to clinical indicators of biofilm. We previously observed that little validation work has been done in this area, and we advocated that efforts should be made to

validate reported signs and symptoms of biofilm in chronic wounds [21]. The findings of and observations made in this Delphi study would appear to lend further support to this call.

Acknowledgements

The authors have nothing to report

Ethics Statement

Ethical approval to carry out this study was granted by the University of Galway Research Ethics Committee (REC). All participants provided informed consent.

REC application reference number: 2023.08.015

Consent

All participants provided informed consent.

Conflicts of Interest

The authors declare no conflicts of interest.

Data Availability Statement

The data that support the findings of this study are available on request from the corresponding author. The data are not publicly available due to privacy or ethical restrictions.

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Table A1 Descriptive statistics for ratings of all 26 signs and symptom items reported to be indicative of biofilm in chronic wounds rated along a 9-point Likert scale of 1 – 3 = <i>not likely to be indicative of biofilm in chronic wounds</i>, 4 – 6 = <i>somewhat likely to be indicative of biofilm in chronic wounds</i> and 7 – 9 = <i>very likely to be indicative of biofilm in chronic wounds</i>. Green shading represents a <i>consensus to include</i> status, and amber shading represents a <i>no consensus</i> status.						
Clinical sign and symptom items	Round 1	Consensus	Round 2	Consensus	Final decision	
A shiny, slimy layer on a non-healing wound surface that persists despite autolytic or enzymatic debridement but can be removed easily with physical removal techniques such as cleansing or mechanical debridement. The surface reforms quickly (1-2 days) in the absence of frequent intervention (for example, cleansing, debridement).	Respondents (n) Mean (SD) score Median (IQR) score 95% CI	65 8.12 (1.63) 9 (1) 7.73 – 8.52	- - - -	Include N/A	Include	

Failure to respond as expected to antimicrobial therapies (for example, systemic antibiotics and/or topical antimicrobials/antiseptics). Infection may re-emerge when treatment is completed.	Respondents (n) Mean (SD) score Median (IQR) score 95% CI	58 7.8 (1.4) 8 (2) 7.44 – 8.15	Include	-	N/A	Include
				-		
				-		
				-		
Infection lasting more than 30 days or persistent cycles of recurrence (waxing and waning).	Respondents (n) Mean (SD) score Median (IQR) score 95% CI	58 7.74 (1.5) 8 (2) 7.4 – 8.13	Include	-	N/A	Include
				-		
				-		
				-		
Poor quality granulation tissue (for example, discoloured, friable, fragile or hypergranulation).	Respondents (n) Mean (SD) score Median (IQR) score 95% CI	75 7.4 (1.9) 8 (2) (6.94 – 7.8)	Include	-	N/A	Include
				-		
				-		
				-		

Wound fails to close or progress to healing despite optimal wound/patient management.	Respondents (n) Mean (SD) score Median (IQR) score 95% CI	58 7.5 (1.92) 8 (2) 7.0 – 8.0	Include	-	N/A	Include
				-		
				-		
				-		
Persistent or prolonged (low-level) inflammation.	Respondents (n) Mean (SD) score Median (IQR) score 95% CI	72 7.4 (1.6) 8 (2.3) 7.0 – 7.72	Include	-	N/A	Include
				-		
				-		
				-		
Wound duration of more than 6 weeks.	Respondents (n) Mean (SD) score Median (IQR) score 95% CI	61 7.3 (1.9) 8 (3) 6.82 – 7.8	Include	-	N/A	Include
				-		
				-		
				-		

Soft tissue deterioration despite antibiotic treatment and/or surgical debridement	Respondents (n)	57	Include	-	N/A	Include
	Mean (SD) score	7.3 (1.92)		-		
	Median (IQR) score	8 (3)		-		
	95% CI	6.75 – 7.74		-		
Signs of local infection (for example, erythema, oedema, serous effusion, new areas of breakdown, pain, etc.)	Respondents (n)	75	No consensus	24	Include	Include
	Mean (SD) score	6.6 (2.22)		7.33 (1.7)		
	Median (IQR) score	7 (3)		7.5 (2)		
	95% CI	6.1 – 7.1		6.7 – 8		
Tunnelling (along tissue structures, degradation along solid structures such as tendon or bone) and/or undermining (erosion of the subcutaneous layer under the wound) edge.	Respondents (n)	68	No consensus	24	Include	Include
	Mean (SD) score	6.4 (2.33)		7.08 (1.61)		
	Median (IQR) score	7 (3)		8 (1.25)		
	95% CI	5.8 – 7.0		6.44 – 7.73		

Presence of slough or fibrin.	Respondents (n) Mean (SD) score Median (IQR) score 95% CI	65	No consensus	24 7.13 (1.7) 8 (2.3) 6.5 – 7.8	Include	Include
		59	No consensus	24 6.8 (2.2) 8 (3) 6.0 – 7.63	No consensus	No consensus
		58	No consensus	23 6.6 (2.2) 8 (3) 5.7 – 7.4	No consensus	No consensus
Favourable response to therapies such as corticosteroids, TNF α inhibitors or multimodal strategies such as physical debridement, cleansing and topical antimicrobial agents and dressings.	Respondents (n) Mean (SD) score Median (IQR) score 95% CI	65	No consensus	24 7.13 (1.7) 8 (2.3) 6.5 – 7.8	Include	Include
		59	No consensus	24 6.8 (2.2) 8 (3) 6.0 – 7.63	No consensus	No consensus
		58	No consensus	23 6.6 (2.2) 8 (3) 5.7 – 7.4	No consensus	No consensus
Extreme tolerance to host immune defence.	Respondents (n) Mean (SD) score Median (IQR) score 95% CI	65	No consensus	24 7.13 (1.7) 8 (2.3) 6.5 – 7.8	Include	Include
		59	No consensus	24 6.8 (2.2) 8 (3) 6.0 – 7.63	No consensus	No consensus
		58	No consensus	23 6.6 (2.2) 8 (3) 5.7 – 7.4	No consensus	No consensus

Increased exudate.	Respondents (n) Mean (SD) score Median (IQR) score 95% CI	75 6.3 (2.4) 7 (3.5) 5.7 - 6.8	No consensus	24 6.54 (1.9) 7 (3) 5.8 - 7.3	No consensus	No consensus
Malodour	Respondents (n) Mean (SD) score Median (IQR) score 95% CI	70 6.3 (2.3) 7 (3) 5.74 - 6.8	No consensus	24 6 (1.83) 6 (2) 5.23 - 6.7	No consensus	No consensus
Negative cultures despite signs/suspicion of clinical infection.	Respondents (n) Mean (SD) score Median (IQR) score 95% CI	61 6 (2.4) 7 (3) 5.41 - 6.6	No consensus	24 7 (2) 8 (3.3) 6.2 - 7.75	No consensus	No consensus

Debris or foreign objects in the wound (for example, pins, mesh, prosthetic devices).	Respondents (n) Mean (SD) score Median (IQR) score 95% CI	87 5.9 (2.62) 7 (4) 5.31 – 6.41	No consensus	25 6.2 (2.43) 7 (4) 5.21 – 7.11	No consensus	No consensus
History of systemic antibiotics in the last 12 months, prior or current use of antimicrobials.	Respondents (n) Mean (SD) score Median (IQR) score 95% CI	58 6 (2.6) 6.5 (3) 5.3 – 6.61	No consensus	23 5.52 (2.5) 5 (4.5) 4.5 – 6.53	No consensus	No consensus
Sepsis	Respondents (n) Mean (SD) score Median (IQR) score 95% CI	67 5.6 (3) 6 (6) 4.84 – 6.3	No consensus	24 6.1 (2.64) 7 (3) 5.03 – 7.14	No consensus	No consensus

Swelling/wound bed with pale oedema.	Respondents (n) Mean (SD) score Median (IQR) score 95% CI	70 5.63 (2.4) 6 (3) 5.1 – 6.2	No consensus	24 6.25 (2.11) 6.5 (4) 5.41 – 7.1	No consensus	No consensus
Wound edge (for example, maceration, non-physiological colour changes of the wound margin, hyperkeratosis).	Respondents (n) Mean (SD) score Median (IQR) score 95% CI	64 5.14 (2.64) 5 (4.3) 4.5 – 6.0	No consensus	24 5.83 (2.5) 6.5 (4.25) 4.9 – 6.82	No consensus	No consensus
Signs of necrotic tissue/eschar.	Respondents (n) Mean (SD) score Median (IQR) score 95% CI	71 5.2 (2.6) 5 (5) 4.6 (5.8)	No consensus	24 5.04 (2.5) 5 (4) 4.04 – 6.05	No consensus	No consensus

Temperature. Sometimes low-grade fever.	Respondents (n) Mean (SD) score Median (IQR) score 95% CI	65 4.91 (2.6) 5 (4) 4.3 (5.5)	No consensus	24 5.2 (1.81) 5 (2) 4.44 – 6.0	No consensus	No consensus
Wound pain/pain at site.	Respondents (n) Mean (SD) score Median (IQR) score 95% CI	69 5.4 (2.1) 5 (3) 5.0 – 5.84	No consensus	24 5.83 (1.5) 5.5 (2) 5.22 – 6.42	No consensus	No consensus
Other patient history indicators (for example, change in the patient's overall wellbeing or quality of life).	Respondents (n) Mean (SD) score Median (IQR) score 95% CI	57 4.81 (2.51) 5 (5) 4.2 – 5.5	No consensus	23 5.04 (2.7) 4 (4.5) 4.0 – 6.14	No consensus	No consensus

Low-level/increased erythema.	Respondents (n)	78	No consensus	24	No consensus
	Mean (SD) score	4.8 (2.31)		5.5 (2.2)	
	Median (IQR) score	4.5 (4)		6 (3)	
	95% CI	4.24 – 5.3		4.63 – 6.4	
SD = standard deviation, IQR = interquartile range, CI = Confidence Interval.					

3.5 Thesis phase 3: Protocol for a clinical study

3.5.1 *Introduction*

This section of the methods and results chapter presents an ethically sound and methodologically robust protocol for a clinical study in which the list of 11 clinical signs and symptoms thought to be indicative of biofilm that resulted from the electronic Delphi consensus study will have their true predictive abilities statistically determined. A sample of consenting patients with lower-limb ulceration secondary to type 2 diabetes will have their wounds assessed by three clinicians for presence or absence of each sign and symptom on the list. A biopsy will then be taken from the wound edge, and this will be tested for presence of biofilm using SEM and a fluorescence staining technique in conjunction with CLSM. The wound assessments will be paired with the biopsy findings, and the sensitivity, specificity positive predictive value and discriminatory power will be calculated for each sign and symptom.

3.5.2 Protocol for a clinical study

The following is a draft of the protocol for the clinical study described in subsection 3.5.1.

Draft patient and clinician participation information leaflets and informed consent forms, a draft data collection form, a draft letter to care providers, a master list for pseudonymising study data, a letter of indemnity from the University of Galway Insurance Services office and approval letters for the study protocol from Galway Clinical Research Ethics Committee can be found in Appendix 3.

A clinical study to validate signs and symptom reported in the literature to be indicative of biofilm in chronic wounds against a gold standard biopsy in a sample of patients with diabetic foot ulceration.

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PhD supervisors: Professor Georgina Gethin, School of Nursing & Midwifery, University of Galway & Professor James P. O’Gara, Microbiology, School of Natural Sciences, University of Galway.

Background

Injuries to the skin typically advance toward healing along a complex and orderly sequence of events comprising haemostatic, inflammatory, proliferative, and remodelling/maturation phases. However, this process is prone to interruption and can become disordered secondary to comorbidities such as chronic venous insufficiency, unresolved pressure, or diabetes, causing oftentimes minor injuries to devolve into chronic wounds which exhibit lengthy periods of stalled or delayed healing. ^[1, 2]

Chronic wounds are commonplace and are a heavy load for both the individual and healthcare systems to bear. Two meta-analyses of three and nine studies from 2019 estimated the pooled prevalence of mixed aetiology chronic skin ulcers to be 2.21 per 1000 population and that of chronic leg ulcers to be 1.5 per 1000 population respectively. ^[3] Their adverse effects on quality of life and wellbeing are well recognized as are their associated costs. ^[4, 5]

Chronic wounds are susceptible to infection, and with infections, poor clinical outcomes can manifest. The risk of hospitalisation in persons with diabetic foot ulceration for example, increases 50-fold with onset of infection, and their risk of undergoing a lower-limb amputation

increases by a factor of 150. ^[6, 7] When we think of infections and bacteria, we may picture single cells free-floating in an aqueous environment or affixed to a solid surface. However, bacterial cells can also aggregate and organise themselves into complex single or multi-species communities that surround themselves in a self-produced polymeric matrix and can attach to a surface. These communities are called biofilms and once established, their co-operative nature and protective matrix render them highly tolerant to adverse environments, host defences and eradication by antibacterial agents. ^[8-10]

The biofilm phenotype is thought responsible for up 80% of all human infections and is estimated to inhabit 60 -78% of chronic wounds possibly more than 90%. Within these wounds they employ complex survival strategies, both inherent to the biofilm and based on biofilm-host interactions that can be harmful to the host. They are thought to be a highly significant contributor to delayed healing after efforts to mitigate other causative factors such as managing underlying systemic disease have been made. ^[11-17]

Currently accepted methods to identify biofilms require advanced microscopy of tissue samples, sometimes in conjunction with fluorescent probes, but these are not readily available to clinicians. ^[8, 18] The standard method for identifying wound pathogens in the clinical setting is microbial culturing. However, culturing can underestimate the full degree of colonisation. This is thought to be due to presence of viable but non-culturable cells – cells in a biofilm that exist in a dormant or slow-growing state and do not form colonies under normal culturing conditions. ^[19, 20]

Wound care clinicians then, tend to fall back on visual cues and clinical signs and symptoms which serve as surrogate indicators to confirm biofilm presence as is common practice for diagnosing wound infections. ^[21] We carried out a scoping review of the literature, ^[22] and have collated a list of clinical signs and symptoms reported to be indicative of biofilm in chronic wounds. We included 111 full-text reports which yielded 830 instances where a visual cue, sign, or symptom for example, “Opaque, loosely attached patches” or “failure to respond to antibiotics” was said to be indicative of biofilm in chronic wounds. This list was refined into 26 statements of clinical signs thought to be indicative of biofilm in chronic wounds and a subsequent electronic Delphi exercise involving an international panel of experienced wound care clinicians further narrowed this list down to 11. ^[23]

It is important to recognise signs and symptoms for biofilm, but it is also important to acknowledge that wound biofilms are difficult to visualise macroscopically, and signs such as wound slough or other visual markers are thought problematic, seen as being overly subjective,

without basis and objective criteria. ^[24] If we can't easily see biofilm in chronic wounds, how do we know if it is there (or gone)? Failure to identify wounds containing biofilm may result in ineffective treatment and delayed healing. ^[15] Conversely, we are concerned that assuming biofilm to be present when it is not may lead to excessive use of treatments, thereby compromising good antimicrobial stewardship. In addition, we wonder if biofilm removal truly helps restore an optimal healing trajectory and if antibiofilm agents which demonstrate efficacy *in vitro*, are effective in real life.

A reliable method to determine biofilm presence in chronic wounds at the bedside may help address these concerns. Clinical prediction rules can be conceptualised as "the combining of relevant clinical findings to calculate a numeric probability of the presence of a specific disorder or likelihood of an outcome, that is, they act as adjuncts to the diagnostic process.", ^[26] and so the objective of this clinical study is to determine the diagnostic capabilities of signs and symptoms reported in the literature to be indicative of biofilm in chronic wounds against a gold standard biopsy in a sample of persons with diabetic foot ulceration.

Research Question

Which of the following clinical signs and symptoms reported in the literature to be indicative of biofilm and voted to be very likely such by an international panel of experienced wound care clinicians in an electronic Delphi exercise, can be confirmed to be so through validation against a gold-standard biopsy in a sample of persons with diabetic foot ulceration?

- Poor quality granulation tissue (e.g. discoloured, friable, fragile or hypergranulation).
- Persistent or prolonged (low-level) inflammation.
- A shiny, slimy layer on a non-healing wound surface that persists despite autolytic or enzymatic debridement but can be removed easily with physical removal techniques such as cleansing or mechanical debridement. The surface reforms quickly (1-2 days) in the absence of frequent intervention (e.g. cleansing, debridement).
- Wound duration of more than 6 weeks.
- Failure to respond as expected to antimicrobial therapies (e.g. systemic antibiotics and/or topical antimicrobials/antiseptics. Infection may re-emerge when treatment is completed.
- Wound fails to close or progress to healing despite optimal wound/patient management.
- Infection lasting more than 30 days or persistent cycles of recurrence.
- Soft tissue deterioration despite antibiotic treatment and/or surgical debridement.

- Signs of local infection (e.g. erythema, oedema, serous effusion, new areas of breakdown, pain, etc.).
- Presence of slough or fibrin/fibrin coating.
- Tunnelling (along tissue structures, degradation along solid structures such as tendon or bone) and/or undermining (erosion of the subcutaneous layer under the wound edge).

More specifically, what is the sensitivity and specificity of each clinical sign and symptom thought to be indicative of biofilm in chronic wounds in a sample of diabetic foot ulcer patients when compared to a gold standard biopsy of wound tissue?

What is the discriminatory power of each sign and symptom when compared to the gold standard biopsy of wound tissue?

What is the positive predictive value of each sign and symptom when compared to a gold standard biopsy of wound tissue? ^[27]

What is the inter-rater reliability of each sign and symptom as estimated by Cohen's Kappa statistic? ^[28]

Plan of investigation

Setting and participants

The setting for this is the Diabetes Day Centre, Hospital 5, St. James's hospital, James's St., Dublin, D08 TYF3. The study aims to recruit 12 patient participants and 3 clinician assessor participants in total with recruitment facilitated by clinical personnel. Fully informed written consent will be obtained from participants prior to engaging in any study related procedures.

Eligibility criteria

We are aware of 4 articles that have taken biopsies from chronic wounds including diabetic foot ulcers for biofilm visualisation/confirmation purposes. ^[25, 29-31]

In a study to visualise and confirm biofilm in DFU, Johani et al. ^[25] took a biopsy post cleansing with 0.9% NaCL from patients with wounds that were not responding to standard care, not healing within an appropriate time frame or presenting with a new acute clinical infection. Malone et al. ^[29] carried out a study in patients with DFU to determine the efficacy of cadexomer iodine on bacterial load and diversity. Biopsies were taken post cleansing with 0.9% NaCL. Sharp debridement was withheld for the duration of intervention administration (seven days). Patients had to have ulcers of greater than six weeks duration that were failing to respond

to standard care. Patients with clinical signs of infection, osteomyelitis, having received topical or systemic antimicrobials two weeks prior to enrolment or a contraindication to cadexomer iodine were deemed ineligible. Fazli et al. [30] took a biopsy before cleansing and surgical preparation of venous leg ulcers (VLU) in their study to examine the distribution of *S. aureus* and *P. aeruginosa*. Eligibility criteria were not reported. Finally, Kirketerp-Møller et al., [31] did not report in a procedure for taking biopsies in their study to elucidate the distribution, organisation and ecology of bacterial in chronic wounds. Participants were selected on the basis of a clinical suspicion of *P. aeruginosa*.

Two of the studies [25, 29] took biopsies post cleansing and one [30] took biopsies pre cleansing. Eligibility criteria were reported had elements that were contingent on study objective, [29, 31] with two requiring that participants have non healing wounds that were not responding to standard care, [25, 29] and one excluding patients with clinical signs of wound infection. [29] One of these [25] was a biofilm visualisation study.

In terms of determining the abilities of a set of clinical signs and symptoms to predict presence or absence of a condition using a gold standard such as a biopsy, an ideal study sample would include both condition positive and condition negative participants in a similar proportion to the prevalence of the condition in the population. Eligibility criteria for such a study should therefore be such that the probability of including both condition positive and condition negative participants is maximised.

If eligibility criteria for this study only permit entry of patients with wounds that were not healing, not responding to standard care or presenting with infection as per Johani et al. [25] and Malone et al., [29] then the probability of excluding participants who may be truly negative for biofilm could be increased. Conversely, if eligibility criteria for this study prevent entry of patients with clinical signs of infection as per Malone et al., [35] then the probability of excluding patients who may be truly positive for biofilm could be increased.

The eligibility criteria for this study thus attempted to maximise the balance of condition positive and condition negative patients in its sample simply by requiring that patients have active diabetic foot ulceration regardless of infection status whilst being mindful of the safety needs of the patient.

Inclusion: Inpatients and outpatients ≥ 18 years of age with type 2 diabetes, capable of providing autonomous consent will be eligible for this study. Participants must have active diabetic foot ulceration (DFU) defined by the International Working Group on the Diabetic Foot (IWGDF)

as “Infection, ulceration or destruction of tissues of the foot of a person with currently or previously diagnosed diabetes mellitus, usually accompanied by neuropathy and or peripheral arterial disease (PAD) in the lower extremity.”^[32]

Ulcers must have a University of Texas Staging System for diabetic foot ulcers score of no more than Grade I, Stage B.^[33]

Exclusion: Participants with blood dyscrasias, venous congestion or extremely vascular wounds in a setting where there is limited access to care or high risk that the biopsy site will not heal will not be eligible for participation in this study.^[34]

In addition, absolute contraindications to epinephrine, that is, untreated hyperthyroidism, and pheochromocytoma will render patients ineligible for entry to this study.

Clinician participants (assessors) must be employed as clinical personnel at the Diabetes Day Centre, St. James’s Hospital, Dublin, and experienced in chronic wound assessment.

Study design and methods.

A cross-sectional study design to determine the inter-rater reliability and validity of 11 clinical signs and symptoms reported in the literature to determine presence of biofilm in the diabetic foot ulcers of inpatients and outpatients patients with type 2 diabetes attending the Diabetes Day Centre, St. James’s Hospital, Dublin.^[35]

Sample size

To our knowledge, no attempts have yet been made to develop a clinical prediction rule for clinical signs and symptoms reported in the literature to be indicative of biofilm in chronic wounds, in particular with our chosen methodology.

We would therefore consider it prudent to carry out this work on a reduced scale and with a considerably smaller sample size than would normally be required for this type of research to assess the feasibility of the methodology and determine its strengths, weaknesses prior to attempting it on a larger, more resource intensive scale.

We have therefore decided to recruit 12 patients from the St. James’s Hospital site.

Three clinical personnel will be recruited at this investigative site to participate in the inter-rater reliability portion of this study.

Study visit procedures

When presenting for routine wound assessments, clinic staff/site study investigators will introduce the study to the patient and ask if they would like to receive information about this study.

If the patient agrees, the study will be verbally introduced, its nature, purpose and associated benefits/risks articulated followed by an offer of the participant information leaflet. The patient will be informed that they may take as long as they require to review the document and discuss with caregivers, family, or other medical professionals, and that a decision to not participate will not lead to penalisation, alteration of treatment nor will it adversely affect their rights as a patient at the clinic in any way.

A similar process will be used to recruit clinical personnel to participate in the inter-rater reliability portions of this study.

If the patient/clinician meets eligibility criteria, and on confirmation of willingness to participate via a signed and dated consent form, it will be re-iterated that they may withdraw from the study at any time without penalisation, alteration of treatment and without risk to their rights as a patient/employee at the clinic being affected.

Demographic information including patient details, that is, age, sex, duration of diabetes, and wound-related information, that is, ulcer location, duration, University of Texas Staging system score and wound dimensions (length X width cms) will be collected on the data collection form (DCF).

Consenting clinical personnel will be asked to provide their medical profession (e.g. doctor, podiatrist, nurse) and number of years of experience.

Post dressing removal, the wound will be covered with a dry gauze dressing and left in place for 10 minutes to standardize conditions for assessing the wound.^[29]

To determine inter-rater reliability, two consenting clinicians will assess the wound against the biofilm signs and symptoms list. Assessments will be done independently. Assessors will place their completed sheets in an opaque envelope labelled “Interrater reliability” along with the date and participant number, which will then be sealed by the attending clinician/assessor.

The attending clinician/main assessor will then assess the wound with the same signs and symptoms list and place the completed sheet in a second opaque envelope labelled “Validation” along with the date and participant number, and seal it.

The participant will receive a local anaesthetic injection of 1 -2% lidocaine with or without epinephrine. Epinephrine is premixed with local anaesthetic (1:100,000, 10mg/ml lidocaine

plus 10mcg/ml epinephrine for a final concentration of 1% or 1:200,000, 20mg/ml lidocaine plus 5mcg/ml for a final concentration of 2%).

The wound will be cleansed with 0.9% NACL and prior to debriding, a 4mm wide x up to 5mm deep punch biopsy will be taken from the wound edge.^[34] The biopsy punch will be rotated approximately 180 degrees clockwise and then anticlockwise while being pushed into the skin until it reaches the appropriate depth. Tissue will be elevated with the aid of forceps or needle, and scissors are used to cut the base. After taking the biopsy, haemostasis will be achieved by applying local pressure with 2 pieces of gauze dressing.

The skin adjacent to the where the biopsy was taken will be marked with a sterile surgical skin marker to highlight the site, and for safety purposes, a photograph of the wound may be taken and kept in the participant's medical chart. The photograph will not be otherwise processed or used as part of the study.

An extra pressure layer (gauze and tape) will be applied over the participant's usual dressing and left in place for 48 hours.

Sample storage

Biopsy samples will be split longitudinally into 2mm sections using a forceps and scalpel.

Samples for fluorescence staining will be fixed in 4% paraformaldehyde.

Samples for electron microscopy will be fixed in 2% glutaraldehyde + 2% paraformaldehyde in 0.1M sodium cacodylate buffer pH = 7.2 (100ml stock).

Samples will be stored at 4°C on site. Once all samples have been collected they will be transferred by courier to the University of Galway for processing and analysis as described below.

Fluorescence imaging^[29-31]

- Samples for fluorescence staining will be fixed in 4% paraformaldehyde.
- Fixed samples will be stored at 4°C at the Diabetes Day Centre, St. James's Hospital until they can be transferred by courier to Microbiology, School of Biological and Chemical Sciences, University of Galway and stored at 4°C prior to further processing.

- For processing, samples will be embedded in optimal cutting temperature compound, snap frozen in liquid nitrogen at Microbiology, School of Biological and Chemical Sciences, University of Galway and stored at -70°C until use.
- Serial sections of 8µm will be cut from the surface, middle and base of the samples with a cryostat, picked up on poly-L-lysine-coated slides and air dried.
- Tissue sections will be fixed in formalin for 10 mins at room temperature.
- Sections will be stained with five fluorescent dyes. Bacterial cell DNA will be stained with either 2µg/ml 4',6-diamidino-2-phenylindole (DAPI) (Thermo Scientific™) or 2µg/ml SYTO-9 (ThermoFisher Scientific) green fluorescent nuclear and chrome counterstain.

Biofilm polysaccharide-type ECM will be stained with either 5µg/ml Wheat Germ Agglutinin (WGA) (Invitrogen™) or 1:2 Calcofluor White Stain (Sigma-Aldrich): PBS while 1:2 Film Tracer™ SYPRO™ Ruby biofilm matrix stain (Invitrogen™): PBS will be used to stain biofilm protein-type ECM.

- Sections will be analysed with confocal laser scanning microscopy.

Scanning Electron Microscopy (SEM)

- Samples for SEM will be fixed immediately by immersing completely in primary fixative* and left at room temperature for 2 hours.
- Fixative will then be removed, and samples will be rinsed twice in 0.2M sodium cacodylate buffer**. Samples will be left in buffer for 5 minutes for each rinse.
- Samples will be kept in 0.2M cacodylate buffer and stored at 4°C at St. James's Hospital until they can be transferred by courier to Microbiology, School of Biological and Chemical Sciences, University of Galway and stored at 4°C prior to further processing.
- Buffer will be removed, and 30% ethanol will be added and left for 15 minutes. This step will be repeated once (samples must not be allowed to dry in air until the whole process is complete).
- The 30% ethanol will be removed and replaced with 70% ethanol and left for 15 minutes. This step will be repeated once (samples can be stored in 70% ethanol overnight or until further processing if necessary).
- The 70% ethanol will be removed and replaced with 90% ethanol and left for 15 minutes. This step will be repeated once.

- The 90% ethanol will be removed and replaced with 100% ethanol and left for 15 minutes. This step will be repeated once.
- The 100% ethanol will be replaced with 100% ethanol.
- Following the final 100% ethanol dehydration step, the ethanol will be removed from the sample and replaced with Hexamethyldisilazane (HMDS) for 2 X 15 minutes.
- The HMDS will be removed from the sample, and the sample will be placed on velin paper in a petri dish with the lid slightly open and left to air dry overnight in a fume hood.
- Samples will then be mounted onto aluminium stubs with a double-sided sticky tab and gold sputter coated.
- Samples will then be ready to be viewed on the SEM.

*EM primary fixative: 2% glutaraldehyde + 2% paraformaldehyde in 0.1M sodium cacodylate buffer pH 7.2 (100 ml stock).

To be performed in a fume hood.

- Weigh out 21.4g of sodium cacodylate powder.
- Dissolve powder in 400ml Millipore grade distilled H₂O.
- Add 42ml of 0.2N hydrochloric acid to the solution and adjust to pH 7.2 (Note: the addition of HCL produces arsenic gas and so this step must be performed in a fume hood).
- Make up the solution to a final volume of 500ml with Millipore grade distilled H₂O.
- Store the solution at 4°C in a clearly labelled screw-top bottle.

** EM buffer: 0.2M sodium cacodylate buffer pH 7.2 (500ml stock solution)

To be performed in a fume hood.

- To a 100ml graduated cylinder, add the following:
 - 8ml of 25% glutaraldehyde (use a 10ml graduated cylinder)
 - 12.5ml of 16% paraformaldehyde (methanol free) (1 ampoule + 2.5ml from another ampoule)
 - 50ml of 0.2M sodium cacodylate buffer.
- Make up the solution to a final volume of 100ml with Millipore grade distilled H₂O.
- Store the solution at 4°C in a clearly labelled screw-top bottle.

Ethanol solutions (500ml of each)

- 100% ethanol: (from Sigma Aldrich catalogue no. E 7023).
- 90% ethanol: 450ml of 100% ethanol + 50ml deionised H₂O.
- 70% ethanol: 350ml of 100% ethanol + 150ml deionised H₂O.
- 50% ethanol: 250ml of 100% ethanol + 250ml deionised H₂O.
- 30% ethanol: 150ml of 100% ethanol + 350ml deionised H₂O.

Data processing and statistical analysis.

Study data will be processed in RStudio. Patient demographics and wound data will be presented descriptively in terms of mean and standard deviation or median and IQR/range.

Using 2 X 2 tables (see below) for each of the 11 signs and symptoms thought to be indicative of biofilm in chronic wounds that describe the number of patients with true positive (TP), false positive (FP), true negative (TN) and false negative (FN), sensitivity or true positive rate (TPR), specificity, discriminatory power, and positive predictive value will be calculated:

- Sensitivity = $TP / (TP + FN)$
- Specificity = $TN / (TN + FP)$

The likelihood ratio (LR) of the sensitivity (TPR), and the false positive rate (FPR) i.e. 1 - specificity will be calculated as a representation of the discriminatory power of each sign and symptom:

- $LR = TPR / FPR$ (i.e. sensitivity / 1 – specificity)

Positive predictive value (PPV) will be calculated as:

- $PPV = TP / (TP + FP)$ ^[29]

Clinical sign/symptom	Biofilm present (as per biopsy)	Biofilm absent (as per biopsy)
Rated present (test +)	True positive (TP)	False positive (FP)
Rated absent (test -)	False negative (FN)	True negative (TN)

Inter-rater reliability of each sign and symptom in the list will be estimated using Cohen's Kappa i.e. observed agreement – expected agreement / 1 – expected agreement. ^[29]

Clinical training

A study initiation meeting in which the study background, procedures and assessment tool will be outlined will be held at the Diabetes clinic to which clinical personnel will be invited.

Consenting personnel will review the signs and symptoms list and gain experience using it with up to 5 patients who are not currently involved in the study and are agreeable to having their wounds assessed. ^[30]

The researcher will receive appropriate imaging and sample preparation training at Microbiology, School of Biological and Chemical Sciences, University of Galway and Anatomy, Imaging & Microscopy, University of Galway.

Ethical considerations and data protection

Ethical approval will be sought through the St. James's Hospital (SJH)/Tallaght University Hospital (TUH) research ethics committee.

No personal identifiers or identifiable information will be captured in data collection sheets. Non-identifiable digit codes will be assigned to each study participant and participating clinical personnel. These codes will be used to label data collection sheets and tissue sample containers. A master list linking participants to digit codes will be kept under secure conditions in the Diabetes Day Centre, St. James's Hospital, Dublin from where it will not be removed. Coded raw data will be kept under secure conditions in Diabetes Day Centre, St. James's Hospital, Dublin. This data will be entered in Microsoft Excel onsite after imaging analyses of tissue samples has been completed (to maintain blinding). The master list, data collection sheets and signed informed consent forms will be kept onsite at the Diabetes Day Centre, St. James's Hospital in a secure location for 7 years after which it will be destroyed. All coded data in Excel will be held on the University of Galway's password protected cloud server (OneDrive) for a period of 7 years.

Tissue samples will be marked with the project title (abbreviated), date of sampling and a participant digit code only. They will be transferred directly from the Diabetes Day Centre, St. James's Hospital, Dublin to refrigeration facilities at Microbiology, School of Biological and Chemical Sciences, University of Galway. No donor genetic data will be captured, and samples will not be used for any secondary research* purposes. All tissue sample material will be destroyed upon completion of the project.

Underlying causes of lower limb ulceration in persons with diabetes include poor glycaemic control, improper foot care, poorly fitting footwear, foot deformities, callouses, peripheral neuropathy, arterial insufficiency, peripheral vascular disease, and impaired resistance to infection.^[36, 37] Biopsies are invasive and consequently there are risks associated with using this procedure in persons with diabetes and foot ulceration. However, there are published studies where biopsies have been taken from diabetic foot ulcers for biofilm imaging purposes.^[25, 29] Potential participants will be assessed for healability of their wounds prior to entry into

this study. If the risk of the procedure is deemed too great to a patient through clinical opinion, that patient will not enter the study.

Individuals who undergo the procedure will be advised to contact the clinic subsequent to the study visit if they have any concerns. They will be closely monitored at subsequent clinic visits, and a letter will be provided to inform community care nurses and/or general practitioners that the individual has recently participated in a clinical study in which a biopsy was taken.

Local anaesthesia will be administered with a hypodermic syringe. Although peripheral neuropathy typically renders the extremities insensate, participants may still experience some pain or discomfort. This potential for pain or discomfort will be outlined in the participant information leaflet and participants will be verbally forewarned before the procedure takes place.

Any invasive procedures come with a risk of infection. All possible steps to mitigate risk of infection will be taken while administering study procedures.

The study procedures are estimated to add approximately 30 minutes to the duration of a routine clinical visit. This will be made clear in the participant information leaflet so that potential participants may factor the time requirements into the scheduling of their day.

It will be made clear to participants that without needing to provide a reason, consent can be withdrawn at any time during the study visit without penalisation, alteration of treatment or adverse effects to their rights as a patient at the clinic in any way. In particular, it will be made clear that the option to decline any study procedure is always available.

If a participant becomes distressed during the study visit, the attending clinician will stop and check as to whether they wish to continue or not. If the person wishes to continue, the attending clinician will proceed while maintaining vigilance for further distress. If the participant continues to show signs of distress, the study visit will be terminated. The attending clinician will stay with the person until they become calm again and will discuss with the identified support person (the attending clinician) the distress and supports available.

If a participant does not wish to continue, the study visit will be terminated, and the attending clinician will stay with the person until they are calm again. The identified support person (attending clinician) will discuss measures necessary to provide adequate support for the individual.

“Secondary research refers to additional research carried out on the data and/or tissue acquired during a (primary) research process. The scope of secondary research (where a new consent process has not taken place) is limited, as indicated in the definition of ‘broad informed consent’.” [38]

“Broad consent for research refers to consent obtained for additional secondary use of biological material and/or personal data for secondary research that has not yet been specifically defined. The scope of the secondary research is limited to the area of research, or purpose of the research project, specified in the original consent. However, when the purpose of the research cannot be fully specified, the researcher must find other ways to ensure that the consent requirements are met, e.g. data anonymisation, dynamic consent, and ensuring transparency.” [40]

Resources

Access to patients, clinical personnel and facilities of the Diabetes Day Centre, St. James’s Hospital, Dublin.

Access to storage and laboratory facilities and equipment at Microbiology, school of Biological and Chemical Sciences, University of Galway.

Access to imaging equipment at Anatomy, Imaging and Microscopy, University of Galway.

Aside from microscopy time, there are no costs anticipated for this study. However, the average study -related costs considering study materials such as dressings, anaesthetic, syringes/needles, punch biopsies, sample containers etc. will be estimated for a single patient in this work and this data will be used to develop budgets for larger study iterations.

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3.5.3 *Interim chapter conclusion*

This chapter has thus far presented three of the five methodological phases comprising this PhD thesis and their results. The scoping review of phase 1 provided a list of clinical signs and symptom items reported in the literature to be indicative of biofilm in chronic wounds. These were funnelled into the electronic Delphi consensus study of phase 2 where they were refined into a shorter list of 11 items which was then forged into a robust protocol for a clinical study in phase 3 designed to allow the true abilities of those items to predict biofilm presence in the chronic lower-limb ulcers of a sample of patients with type 2 diabetes. The reader is now invited to proceed to the next sections of this chapter in which phase four, the experience of finding an appropriate investigative site for this study and working through the regulatory, ethical and data protection systems that grant approval for it to proceed there will be presented. This will be followed by phase five, in which an effort to develop an imaging procedure to identify biofilm in tissue samples and its outcomes will be documented.

3.6 Thesis phase 4: Investigative site access

3.6.1 Introduction

This chapter/section takes a more narrative approach than previous sections as it sets out the extraordinary steps that were taken to gain access to the clinical sites in order to take biopsies for research purposes. The process could not commence until the scoping review and Delphi were completed and thus the timepoint in the PhD process was unfortunate. It also takes a short delve in the history of clinical research that has laid the foundation for the current regulatory processes and justifies the current measures that are in place to protect the rights and safety of individuals. The researcher will present the level of communication, presentations and applications that were required to try to gain approval. This could not have been foreseen at an earlier timepoint in the PhD process as will be described, due to changes in governance structures and processes and approvals at two clinical sites.

Clinical research strives to produce knowledge that will promote understanding of human health and disease and find innovative ways of preventing, treating, detecting and diagnosing it (Institute of Medicine Clinical Research Roundtable, 2002). Fundamental to clinical research is the clinical study wherein human volunteers or participants are studied, usually at a clinical study site such as a hospital, research centre or medical facility to provide data from which this knowledge is derived (Iliomad Health Data, 2024).

Research involving human participants comes with inherent risks, and in addition to taking measures to ensure successful execution of a study, measures must be taken to protect the well-being and safety of participants and to ensure that safe, appropriate environments are provided for their care (Lee et al., 2012).

Unfortunately, the rights of research participants to be treated in a safe and dignified manner or the need to conduct careful clinical research have not been consistently recognised and there are tragic examples in recent history where abuses of those rights and neglect of that need have occurred (University of Missouri – Kansas City, Research & Innovation). Three examples in particular will be described in the following paragraphs: 1) Atrocities committed in the name of medical research by Dr. Josef Mengele in the Auschwitz-Birkenau concentration camp during World War two, 2) The Thalidomide scandal that arose in the 1960s and 3) the Tuskegee syphilis study that was exposed in 1972.

During World War two, Dr. Josef Mengele, notorious for condemning prisoners in Auschwitz-Birkenau concentration camp to the gas chambers was responsible for carrying out medical experiments into the cause and treatment of noma – a disfiguring disease affecting facial features and external genitalia in Roma and Sinti children interned at the camp (Halioua and Halioua, 2021). He ordered that all children with noma be hospitalized in a barracks designated the ‘Noma ward’, coerced prisoner doctors into collaborating in the research and treated the children with a combination of sulphanilamido-ethyl thiodiazole and nicotinic acid (Halioua and Halioua, 2021). The children were photographed and sketched at different stages of the evolution of the disease and the bodies of dead children were sent for post-mortem. Mengele eventually murdered any remaining children in the Noma Ward on the night of 2nd – 3rd August 1944 (Halioua and Halioua, 2021). He also participated actively and with an absolute disregard for human life and ethical principles in ophthalmic research, collaborating with genetic researchers at the Kaiser-Wilhelm Institute in Berlin on an eye colour protocol to demonstrate racially-determined hereditary differences in iris structure and ‘ostensibly to “cure” heterochromia’ (Halioua and Marmor, 2020).

In the 1950s Thalidomide was developed for use as a sedative by Chemie Grünenthal GmbH. In 1956 it became licensed for over-the-counter sale based on limited animal and human (with mixed results) testing and was additionally indicated for conditions such as colds, flu and pneumonia (Science Museum, 2019, Cawthon, 2023). It was also indicated for relieving symptoms of nausea and morning sickness in pregnancy without adequate clinical research, because at the time it was not established that drugs could pass through the placental barrier and adversely affect a foetus in the womb (Science Museum, 2019). In 1961, an Australian obstetrician published a letter in *The Lancet* reporting that he had observed “multiple severe abnormalities” in newborns whose mothers had taken the drug and it was subsequently withdrawn from the market (Science Museum, 2019, Stafford, 2018). However, by this time the damage had been done, and it is estimated that over 10,000 babies around the world were affected by the drug and half died within months of being born. Those that survived live permanently with the adverse effects of the drug as do their families (Science Museum, 2019).

In 1972 the US public Health Service (USPHS) conducted ‘Tuskegee syphilis study of Untreated Syphilis’ was made public based on information from an epidemiologist employed by the agency (Tiewsoh, 2025, Gardner and McClelland, 2025). The aim of the study was to evaluate the progression of untreated syphilis on the body and the story revealed that over a 40

year period, 600 African-American males from Tuskegee, Alabama (399 of whom were infected with syphilis) described as “illiterate” were recruited with the promise of free, expert medical care and burial insurance but without an informed consent process (Tobin, 2022, Tiewsoh, 2025). Those confirmed as having syphilis were not told of their diagnosis and were denied treatment with penicillin, whose efficacy in treating syphilis had been established at this point (Gardner and McClelland, 2025, Tiewsoh, 2025). Participants were also led to believe that a lumbar puncture procedure for obtaining cerebrospinal fluid was a treatment and told that vitamin tonics and aspirin administered during the study were effective treatments for their disease which was described as “bad blood” – local vernacular for multiple ailments (Tiewsoh, 2025, Tobin, 2022). Ultimately, 28 participants died from syphilis, 100 died from complications thereof, 40 spouses were infected and 19 children were born with congenital syphilis (Gardner and McClelland, 2025, Tobin, 2022).

Subsequent to the revelation of these and other dark occurrences, steps were taken to develop and implement guidelines and regulatory processes to guarantee that patients of the future would be able to engage with clinical research in an informed, dignified and protected manner. The next paragraphs will describe some of those steps and outline the resulting codes and documentation.

At the end of 1946, a US Military tribunal initiated criminal proceedings against 23 German doctors and administrators for participating willingly in war crimes including medical experiments performed on concentration camp prisoners without consent (United States Holocaust Museum). An argument of the defence was that no international law or informal statement differentiated between legal and illegal human experimentation and this propagated a six-point memorandum, later revised into ten points, defining legitimate medical research which became known as the Nuremberg Code (United States Holocaust Museum). In essence, the code stipulates that the benefits of research must outweigh the risks and that voluntary consent of the human participant is absolutely essential. This document does not carry any legal weight but it has informed numerous international ethics statements (United States Holocaust Museum, University of Missouri, Kansas City, Research & Innovation).

In 1959, a senator from Tennessee named Estes Kefauver began lobbying for reform in the pharmaceutical industry in the United States, focusing predominantly on the issue of pricing

(Higby, 2022). The senator was facing opposition from colleagues with ties to the pharmaceutical industry while attempting to amend the 1938 Food and Drug act, but witness testimonies including that of a Johns Hopkins doctor who had observed the adverse effects of thalidomide were beginning to garner support for his case (Cawthon, 2023). His proposal for pharmaceutical industry reform and regulation was before Congress in July of 1962 when the global scope of the thalidomide tragedy broke in the news, and following in the wake of public outcry, the Kefauver-Harris amendment was enacted in October of that same year (Cawthon, 2023). This new law required that marketing approval of drugs in the US be contingent on their safety and effectiveness, demonstrated through rigorous animal testing and closely-monitored clinical trials (Cawthon, 2023).

In 1964, The 18th General Assembly of the World Medical Association (WMA) adopted the Declaration of Helsinki Ethical Principles for Medical Research Involving Human Subjects, also in response to recent global atrocities and scandals (World Medical Association, 2013, Wise, 2024). The Declaration has international scope and comprises 37 ethical principles and rules intended to guide medical research (Wise, 2024). The primary concerns addressed in the Declaration are that 1) biomedical research involving human subjects should be based on the findings of laboratory and animal experimentation, 2) that research protocols should be subject to review by an independent committee, 3) that informed consent from prospective participants is required, 4) that risks associated with research should not outweigh anticipated benefits and 5) that research should only be conducted by appropriately qualified personnel (University of Missouri-Kansas City, Research & Innovation).

In July of 1974, as a direct response to the Tuskegee Syphilis study, President Richard Nixon signed the National Research Act into law (Rothstein and Wolf, 2024, University of Nevada, Division of Research). This act gave rise to the National Commission for the Protection of Human Subjects of Biomedical and Behavioural Research whose role was to identify fundamental ethical principles to support the conduct of biomedical and behavioural research involving human participants (University of Nevada, Division of Research, Rothstein and Wolf, 2024). The Commission was also tasked with creating guidelines to ensure that research would be conducted in accordance with those principles (Rothstein and Wolf, 2024, University of Nevada, Division of Research). Its best known work was the Belmont Report, broader in scope than previous documents and codes and written with the intent of being easier to interpret and apply, being better capable of covering complex situations and being able to mitigate conflicting

elements arising amongst those codes (Rothstein and Wolf, 2024, Czubaruk, 2019). The code established basic principles of 1) Respect for persons, 2) Beneficence and 3) Justice (University of Nevada, Division of Research). The first principle requires that individuals are seen as autonomous entities and thus entitled to choose what may or may not happen to them as participants in research; those with diminished autonomy must be protected (University of Nevada, Division of Research). This principle is accounted for in the consent process which must be informed, understood and of a voluntary nature (University of Nevada, Division of Research). The second principle requires that human participants be protected from harm and that any research should maximise potential benefits and minimize potential harms (University of Nevada, Division of Research). To fulfil this principle a research project should be assessed for the nature and scope of potential risks and benefits in a systematic manner (University of Nevada, Division of Research). The third principle requires that the risks and benefits of research must be distributed fairly, that is, persons should be included in research based on their connection to the research question and not because of vulnerability in terms of race/ethnicity, socio economic status or institutional confinement for example, nor should they be excluded from research for the same reasons (University of Nevada, Division of Research, Czubaruk, 2019). This principle also requires that the results of research developed from public funds be made available for all (Czubaruk, 2019).

In 1990 The European Commission established The International Council on Harmonization Technical Requirements for Registration of Pharmaceuticals for Human Use (ICH) project with a mission to promote public health by bringing pharmaceutical regulatory authorities of Europe, Japan and the United States together with industry experts to create common scientific and technical standards to guide registration and authorization of pharmaceutical products (Lindström-Gommers and Mullin, 2019, Wandile and R., 2017). In 1996 the ICH released the Good Clinical Practice (GCP) guidelines which provide an ‘international, ethical, scientific and quality standard for the conduct of trials that involve human participants’ and provide counsel for ‘designing, conducting, performing, monitoring, auditing, recording, analysing and reporting (these) trials’ through 13 principles (Table 3) (Wandile & Ghooi, 2017, International Council for Harmonisation, 2025).

Codes and guidelines such as The Nuremburg code, The Declaration of Helsinki and The Belmont report form the basis for ICH GCP and since their inception they have become ‘pillars

of successful global drug development which we have today’ (Wandile and R., 2017, University of Missouri-Kansas City, Research & Innovation).

Table 3. Core principles of the International Conference on Harmonisation Good Clinical Practice (GCP) (Vijayananthan and Nawawi, 2008).

1. Ethics Clinical trials should be conducted in accordance with the ethical principles that have their origin in the declaration of Helsinki, and that are consistent with GCP and the applicable regulatory requirement(s)
2. Trial risk vs. benefit Before a trial is initiated, foreseeable risks and inconveniences should be weighed against anticipated benefit for the individual trial subject and society. A trial should be initiated and continued only if the anticipated benefits justify the risks.
3. Trial participants The rights, safety and well-being of the trial subjects are the most important considerations and should prevail over interest of science and society.
4. Information on the medicinal product The available non-clinical and clinical information on an investigational product should be adequate to support the proposed clinical trial.
5. Good quality trials Clinical trials should be scientifically sound, and described in a clear, detailed protocol.
6. Compliance with the study protocol A trial should be conducted in compliance with the protocol that has received prior institutional review board (IRB)/independent ethics committee (IEC) approval/favourable opinion.
7. Medical decisions The medical care given to and medical decisions made on behalf of subjects should always be the responsibility of a qualified physician or, when appropriate, of a qualified dentist.
8. Trial staff Each individual involved in conducting a trial should be qualified by education, training, and experience to perform his or her respective task(s).
9. Informed consent

Freely given informed consent should be obtained from every subject prior to clinical trial participation.
10. Clinical trial data All clinical trial information should be recorded, handled and stored in a way that allows its accurate reporting, interpretation and verification.
11. Confidentiality The confidentiality of records that could identify subjects should be protected, respecting the privacy and confidentiality rules in accordance with the applicable regulatory requirement(s).
12. Good Manufacturing Practice Investigational products should be manufactured, handled and stored in accordance with applicable Good Manufacturing Practice (GMP). They should be used in accordance with the approved protocol.
13. Quality assurance Systems with procedures that assure the quality of every aspect of the trial should be implemented.

GCP = Good Clinical Practice, IRB = Institutional Review Board, IEC = Independent Ethics Committee, GMP = Good Manufacturing Practice

Finally, in May of 2018 the General Data Protection Regulation (GDPR) came into effect in the European Union (EU) (Office of Government Procurement, 2021). This regulation arose out of concerns about the privacy, accuracy and further use of personal data by public and private organisations (Citizens Information, 2023). Data protection law defines personal data as information that relates to or can identify an individual either alone or together with other available information in terms of three entities: 1) the data controller, that is, the organisation/company holding or using personal data, 2) the subject, that is, the individual whose data is held by the data controller and 3) data processors, that is, other persons, organisations or companies who process data on behalf of the data controller. Any actions carried out on personal data including storing it is known as data processing (Citizens Information, 2023). Under data protection law an individual must be informed of what the data controller intends to do with personal data and is entitled to have their personal data protected, used in a fair and legal manner, made available on request and also corrected on request (Citizens Information, 2023). Data controllers can only use or keep data when there is a lawful reason of which six are specified: 1) The data subject has freely and explicitly given informed

consent, 2) Data processing is necessary to carry out a contract to which the data subject is party, 3) Data processing is necessary for the controller to meet a legal obligation, 4) Data processing is necessary to protect the data subject's vital interests or the vital interests of another individual, 5) Data processing is necessary to perform a task that serves the public interest, 6) Data processing is necessary in the legitimate interests of the processing organisation and does not conflict with the data subject's rights (Citizen's Information, 2023).

The Data Protection Commission (DPC) oversees protection of personal data in the EU, and in addition to dealing with complaints about data breaches under the law it monitors organisations to ensure that they adhere to GDPR and other data protection legislation (Citizen's Information, 2023). To manage data protection at the organisational level, designated Data Protection Officers (DPO) must be appointed where 1) data processing is carried out by a public authority or body, 2) core activities of the data controller or processor comprise processing operations which need regular and systematic monitoring of data subjects on a large scale or 3) the core activities of the controller or processor consist of processing special categories of data (for example, data revealing racial/ethnic origin or political opinions) or personal data relating to criminal convictions and offences on a large scale (Citizen's Information, 2023, Data Protection Commission).

In the University of Galway, as with all institutions in the Republic of Ireland that engage in clinical research, GCP guides the conduct of clinical trials from an ethical and scientific quality perspective. There are offices, administrative personnel and procedures in place to ensure that all clinical research carried out at the institution falls within the remit of GCP. In addition all researchers at the university involved in clinical studies are required to have GCP training (University of Galway Clinical Research & Development Office (CRDO)b).

In the EU, clinical trials may also fall under legal governance of The Clinical Trial Regulation (CTR), the Medical Device Regulation (MDR) or the *In Vitro* Diagnostic regulation (IVDR) (Central Committee on Research Involving Human Subjects (CCMO), TÜV SÜD, European Commission). The CTR serves to harmonise the rules for conducting clinical trials in the EU and applies to controlled studies involving human participants to determine the safety and effectiveness of investigational medicinal products (IMPs) (European Commission). The Medical Device Regulation comprises rules for the submission, assessment and conduct of clinical trials of medical devices, and the IVDR is the regulatory legislation for obtaining

approval to market *in vitro* diagnostic devices (Central Committee on Research Involving Human Subjects (CCMO), TÜV SÜD).

Furthermore, the University has a designated Data Protection Office whose role as per article 39 of GDPR is to 1) ‘inform and advise the controller or the processor and the employees of their obligations under data protection law, 2) to monitor compliance of the organisation with all legislation in relation to data protection, including in audits, awareness-raising activities as well as training of staff involved in processing operations, 3) to provide advice on Data Protection Impact Assessments (DPIAs), 4) to act as a contact point for requests from individuals regarding the processing of their personal data and the exercising of their rights and 5) to cooperate with the DPC and act as their organisation’s contact point for the DPC on issues relating to processing’ (University of Galway, Corporate & Legal Affairs, 2023)

This chapter will document the experiences of the researcher along with an outline of the timelines involved while navigating the ethical, regulatory, data protection and logistical steps leading to acquisition of the necessary documentation and approvals for a clinical study of this nature, pave the way for access to a suitable investigative site and lay the foundations for its successful execution.

A new map will be presented at the end that lays out a streamlined pathway learned through this process that will guide an investigator to successfully complete the necessary steps required to allow regulated and timely execution of the clinical protocol outlined in subsection 3.5.2 in the future.

3.6.2 Initial clinical study preparations

3.6.2.1 Merlin Park Podiatry clinic

In January of 2024 preparations to pave the way for the clinical aspect of this research work began with an email to PhD faculty members and to clinical personnel at the state-of-the art Podiatry Clinic, Unit 3, Merlin Park University Hospital, Galway requesting approval to engage with it as an investigative site for this study. The €800,000 clinic, unique to Ireland opened in 2010 with the aim of providing ‘a high standard of evidence-based podiatric care to the whole population of Ireland and to develop a national resource for healthcare providers by becoming an internationally recognised centre for podiatry care, education and research’ (O'Connor, 2010). Referrals to the clinic include those from consultant-led services such as endocrinology, gerontology, rheumatology and orthopaedics and from Health Services Executive (HSE)

primary care teams, and it is capable of managing 7,000 clients at any one time with up to 25,000 appointments in a calendar year (O'Connor, 2010). In terms of facilities it has three multi-clinic rooms, four single treatment rooms, an outreach prosthesis clinic, a plaster room and a biomechanical suite to carry out gait analysis, pressure analysis and posture assessment (O'Connor, 2010).

However, it was revealed after the start of this process that the clinic had been temporarily stood down and all outpatients with active diabetic foot ulceration were being seen in podiatry-led clinics at the Diabetes Centre, University Hospital Galway (UHG). No date for a resumption of services at the Merlin Park Clinic was available and it was thus decided to approach the Diabetes Centre and request their assistance instead.

3.6.2.2 Diabetes Care Centre, University Hospital Galway

A meeting with a consultant from the Diabetes Centre at UHG and PhD faculty (primary PhD supervisor) to discuss study design and logistics was held online in mid-January of 2024. Attendees were presented with an outline of requirements in terms of participants/sample size, clinic personnel awareness of the study, training requirements and study visit materials such as biopsy kits and local anaesthetic. The anticipated procedure for consenting prospective participants was then described in stepwise fashion as were the anticipated procedures expected to take place during a study visit for data capture and participant care. Finally, other concerns were presented, namely, whether biopsies should be taken pre or post debridement (for safety reasons) and indeed if a biopsy should be taken at all and if not, could alternative options such as curettage, wound swabs or wound blotting be considered instead.

Meeting attendees expressed satisfaction with the proposed consent and study visit procedures and the consultant gave his support to run the study at the clinic and initial approval to take biopsies from their diabetic foot ulcer patient population. They also recommended convening a meeting at the Diabetes Centre where approval to carry out the study could be sought from clinic personnel including nursing, podiatry, tissue viability, administrative personnel and consultants, and their expertise sought in regard to study logistics and methodological procedures.

This meeting took place at the Diabetes Clinic in February of 2024. Attendees were presented with a background and rationale for the study, a summary of the findings of the scoping review, interim findings of the electronic Delphi work, a list of requirements for the study and an outline of the consent and data capture procedures of a study visit. Patient safety concerns (for example,

eligibility criteria), general concerns (for example, study supplies, immediate consent, biopsy location/depth etc.) and a rationale for taking a biopsy as opposed to an alternative means of sampling wound tissue were presented. The feedback from meeting attendees was supportive and it was decided that the study could go ahead at that site with the methodology as presented. However, because the punch biopsy procedure was not standard practice at the clinic it was recommended that other hospital departments including Microbiology, Histopathology and Dermatology be consulted and brought on board if necessary.

A formal 'application for a research project' form was subsequently completed and submitted to the Diabetes Centre to formalise the arrangement.

3.6.2.3 Galway Clinical Research Ethics Committee

During March of 2024 a document package for the study was prepared for submission to Galway Clinical Research Ethics Committee (GCREC) that included a completed local committee checklist and signatory page, a completed GCREC application form, a study protocol, participant information leaflets (PIL) and informed consent forms (ICF), data collection forms (DCF), a sample letter for care providers informing them of their patients' participation in the study and that wound biopsy was taken and a signed and dated curriculum vitae of the principal investigator (PI).

The submission was made in April of 2024, and approval to proceed with the study was granted in the following June after a request by the board to refrain from storing data on an encrypted universal serial bus (USB) key was addressed.

3.6.2.4 Dermatology Department, University Hospital Galway

To resolve the concerns raised at the presentation of the study to Diabetes Centre personnel around taking biopsies at that clinic, a meeting with Department of Dermatology personnel at UHG was held in late June of 2024. Some small changes to the biopsy procedure outlined in the study protocol were recommended (for example, to change the diameter of the biopsy from 3mm to 4mm and the depth from a maximum of 10mm to a maximum of 5mm, and to use local compression rather than sutures to facilitate biopsy site closure), and most importantly it was advised that the biopsy procedure could be performed at the Diabetes Centre by any clinical personnel with training provided by the Dermatology Department if necessary.

A Diabetes Centre consultant offered to carry out the biopsy procedure on consenting participants in the study and an amendment to the protocol was made and submitted to GCREC for approval.

3.6.2.5 Other logistical steps

During this period other issues such as ordering study supplies from the hospital and invoicing procedures between the hospital and University, organising secure storage facilities for study documentation at the Diabetes Centre and the transport of biopsy samples from the Diabetes Centre to storage facilities at the University of Galway were addressed.

3.6.3 Unforeseen circumstances

In August and September of 2024 however, two events presented that warranted a significant change in direction with respect to setting up an investigative site for this study and creating a pathway of access to it.

Firstly, the Chair of GCREC stepped down and all committee business was suspended pending filling of that vacancy (the amendment to this study's protocol did not receive approval until mid-December of 2024) and secondly, the Diabetes Centre informed the study group that due to logistical issues they were forced to suspend the activities of all research projects currently under way there.

3.6.4 A solution and secondary study preparations

3.6.4.1 The Diabetes Day Centre, St. James's Hospital, Dublin

A research colleague based in the Diabetes Day Centre at St. James's Hospital, Dublin was subsequently contacted, and she offered to collaborate, granting access to patients, clinical personnel and facilities to host the study, and the regulatory and ethical approval process between St. James's Hospital and the University of Galway was initiated in October 2024.

At this point in time such an inter-institutional process was unknown territory. Not quite knowing where to begin it was thought best to start with something familiar and so a submission to the St. James's Hospital (SJH)/Tallaght University Hospital (TUH) Joint Research Ethics Committee (JREC) was begun. Access to this committee is gained through an online Ethics Review Manager platform. Once registered with the platform, users receive access to a work area that allows multiple research projects to be managed.

3.6.4.2 St. James's Hospital Ethics application

The online SJH/TUH Research Registration and JREC form is lengthy and additional required documentation included letters of approval from other RECs for multi-site studies (despite the

issues around the Diabetes Centre at UHG participating in this study, it had not been completely put aside as a potential investigative site at this point and so the letter of approval from GCREC was included.), a study protocol, PILs, ICFs, CRFs, a signed PI curriculum vitae, a sample letter for care providers informing them of their patients' participation in the study and that a wound biopsy was taken, a certificate/letter of indemnity from the University of Galway and a dataflow diagram illustrating how data is processed throughout the lifecycle of the study from collection to deletion. Additional documentation included the clinical master list linking participants to their study number and a copy of the University of Texas staging system for assessing DFU.

The submission package was completed by mid-November of 2024. However, a submission to SJH/TUH JREC will not be accepted for review until an SJH Research and Innovation (R&I) form has been completed (this requirement presents at the very end of the SJH/TUH Research Registration and JREC form). Attempts to locate this form including contacting the SJH R&I office initially proved fruitless because during November and December of 2024 the office was in the process of updating their system to incorporate the form on the review manager platform. The form became available in early January of 2025 and was completed soon after. Documentation requested with the form included letters of approval from other RECs, PILs, ICFs, a dataflow diagram, a CRF, a data sharing agreement (DSA) and/or a joint controller agreement (JCA) to allow transfer of materials and data between institutions and if appropriate, a Clinical trial agreement (CTA).

Signed non-disclosure agreement and invitation for vetting by An Garda Síochána forms were also required for non SJH employees who may be present at the investigative site at any time. The SJH/TUH JREC does not have submission deadlines. The full REC holds meetings ten times in a year and reviews six to ten applications at each meeting (Tallaght University Hospital, SJH/TUH Joint Research Ethics Committee, 2022). Conversely, GCREC holds five meetings every year. Each meeting has a submission deadline scheduled approximately one month in advance and if an application is submitted on or before a deadline it will be reviewed at the next scheduled meeting.

The submission for this study to SJH/TUH JREC is ongoing pending inclusion of inter-institutional agreements. Furthermore, given that there are no submission deadlines for REC meetings it cannot be predicted exactly when the submission will be reviewed.

While the ethics submission for SJH/TUH JREC was being prepared, work also commenced in regard to collating the necessary regulatory documentation from the University of Galway.

3.6.4.3 University of Galway Data protection Office

Contact was first made with the University's DPO in early November of 2024, and a consultation meeting was scheduled for mid-November to address queries around GDPR procedures, institutional accountability, data breach policy, necessary agreements for multi-site studies and establishing institutional roles in terms of GDPR entities, that is, data controllers and/or data processors. It was suggested that institutions be viewed as joint data controllers ('where two or more controllers jointly determine the purposes and means of processing data') (GDPR Hub, 2025) and that a joint controller agreement between institutions was most appropriate. The officer also advised that a data protection impact assessment screening checklist be completed and if the answer is yes to any of the questions on this form, then a DPIA form must be completed and submitted. The study intends to capture health-related data from consenting participants which constitutes 'sensitive data, or data of a highly personal nature' on the screening checklist thus warranting a data protection impact assessment.

Work began on this form during the last week of November 2024, and it was submitted to the DPO in mid-February of 2025. The form was first reviewed by the DPO in mid-March of 2025, and a meeting was held in the first week of April 2025 to address their queries. The DPIA form was subsequently revised and resubmitted in the second week of April 2025 along with a copy of the study protocol, PILs, ICFs and drafts of a DSA and a Materials Transfer Agreement (MTA) between St. James's Hospital and the University of Galway. Feedback on the forms was received in mid-May of 2025, revisions were made accordingly, and all forms were resubmitted to the DPO during the first week of June 2025. A follow-up email was sent to the DPO during the third week of July 2025, and a response was received with further feedback and suggestions for revisions at the end of the following August.

3.6.4.4 University of Galway Clinical Research & Development Office

During the third week of November 2024 the Clinical Research and Development Office (CRDO) was contacted. The CRDO comprises clinical research management staff from the University of Galway and Saolta University Healthcare Group (SUHG) and is charged with providing the structures necessary to deliver institutional oversight and approval in terms of clinical research, support for researchers and research activity and with promoting and disseminating research outputs. Its primary objective is to provide direction for clinical research that involves patients, staff and/or infrastructure of Saolta and the University of Galway (University of Galway Clinical Research & Development Office (CRDO)a). A meeting with

the CRDO seeking guidance for this study was held during the last week of November 26th, 2024. In this meeting it was established that the study should be registered with the CRDO on the University of Galway International Research Information System (IRIS) and that an online ‘New Study Clinical Research and Development Office Contract Review Application Form’ be completed. Documents associated with this form include a draft Clinical Trial contract/agreement, a copy of the study protocol, ICFs, PILs, a copy of an insurance certificate, REC approval letters, a completed DPIA, a sponsor/HSE DPO opinion letter, a copy of a study budget if applicable. A joint collaboration agreement between Galway University Hospital, St. James’s hospital and the University of Galway was recommended in terms of GDPR as was a material transfer agreement for clinical samples (The Diabetes Centre at UHG was still being kept in mind as a potential investigative site at this point). The office also advised contacting the university Insurance services office to determine the status of the study in terms of indemnity.

3.6.4.5 University of Galway Insurance Services Office

In the final week of November 2024, the Insurance Services office was contacted to verify that the study would be covered under university indemnity policies. The office reviewed a copy of the completed SJH/TUH research registration and JREC form plus all documentation submitted to GCREC (sub-subsection 3.6.2.3) and their subsequent approval letter. In mid-March of 2025, the office confirmed that the study would be covered by the University Life Science Practice Insurance policy. This policy provides elements of cover such as public liability, legal liability, errors and omissions etc. Insurance services cover patient focused research/clinical research that are not regulated studies or those falling under clinical trials with medicinal products or clinical investigations with medical devices (Central Committee on Research Involving Human Subjects (CCMO)).

3.6.4.6 University of Galway Institute for Clinical Trials

In the final week of November 2024, the Research Office legal team was also contacted with a request for help. The team recommended engaging with the CRDO (which had already been done) and asked for confirmation of membership with the Institute for Clinical Trials (none), a University of Galway initiative with the aims of bridging the gap between research and real-world healthcare, developing partnerships and strategic alliances with industry, innovating research methodologies, providing research education and having meaningful influence on

policy, guidelines and practice (University of Galway Institute for Clinical Trials). In subsequent correspondence from mid-December of 2024 the Research Office advised seeking help from the Institute and provided contact details of the Director of Operations. The Institute was contacted in early January 2025, and a meeting was scheduled for the first week of February 2025 with the Trials Accelerator group at the Institute. This group primarily provides support in the initial phases of clinical trial planning including connection with appropriate methodology experts at the institute, assistance with study design including regulatory pathways and trial methodology, and initial trial activities such as drafting protocols and ethics submissions.

Much of the groundwork for the study had already been laid down – a protocol, PILs and ICFs had been developed, approval from one REC had been granted, a submission to SJH/TUH JREC was underway and headway was being made in terms of inter-institutional agreements and regulatory affairs. The accelerator group confirmed much of the information received from other offices in the university and offered their support for any forthcoming regulatory endeavours. They clarified the status of the study in terms of sponsorship by the University and CTR/MDR (See subsection 3.6.1). Sponsorship by the institution in the context of CTR/MDR entails: legal responsibility for initiating, managing and financing the study; responsibility for regulatory compliance and ensuring that the study adheres to all applicable legislation including CTR, MDR, IVDR and ICH-GCP; conducting a risk assessment of the study and determining the level of oversight necessary; ethical oversight; data management and accepting responsibility as the data controller for the study; insurance and indemnity; quality assurance and integrity of study data and oversight of all delegated activities; support and guidance for researchers with protocol development, ethics submissions and regulatory approvals; training, for example in GCP; establishing a governance and management structure to ensure that sponsorship decisions are well-informed, sustainable and aligned with institutional strategies. While the University will support this study in terms of the factors outlined above, it does not fall within the remit of CTR/MDR and so a formal initiation of the process of sponsoring a clinical trial by the University of Galway, achieved through submission of a Clinical Research Study Registration Form (CSRF) does not need to take place.

The group also offered to help with obtaining signatures of appropriate university representatives for all forms and agreements, recommended following St. James's hospital's lead in terms of inter-institutional documentation and they also advised contacting SJH research

finance management to determine if there would be any study-related costs to take into consideration.

A subsequent meeting with the DPO and the Trials Accelerator group at the Institute for Clinical Trials (described below) in the second week of March 2025 established that this PhD student and University of Galway based study PI, as initial creators of the protocol and responsible for data analysis, constitute data controllers on behalf of the University of Galway who is the study sponsor (although not in the context of CTR/MDR). St. James's hospital will be providing access to patients, facilities and handling data collection and so should be considered to be a separate data controller. A DSA would therefore be most appropriate to cover data protection between both institutions. At this point the Diabetes Centre at UHG was no longer considered to be a potential investigative site for this study.

This PhD project needed access to an investigative site, its patient population, that is, persons with type 2 diabetes exhibiting active lower-limb ulceration, clinical personnel and facilities, that is, a podiatric treatment room where wound assessments could be carried out and biopsies of wound tissue safely taken if it were to meet its responsibility to attempt to determine if a study to investigate the statistical potential of clinical signs and symptoms reported in the literature to predict biofilm in chronic wounds was feasible. It was anticipated that the Diabetes Centre at University Hospital Galway would facilitate this PhD project, but for logistical reasons it found itself in the position of having to suspend all research activities happening there. This was an unforeseen, untimely and serious challenge to this project's needs and ability to meet that responsibility, but a solution presented in an offer by a colleague in the Diabetes Day Centre at St. James's Hospital, Dublin to host the study there. Navigating the regulatory, ethical and data protection systems that provide approved access to this site is proving to be a lengthy process and is still not complete. Furthermore, the colleague who initially offered support at St. James's Hospital has left their position and therefore one may have to start again to gain the confidence of another person to conduct the study.

This change in direction of this PhD project, although proving cumbersome and lengthy has resulted in an unexpected and valuable research output. A novel, first-of-its kind guidance document and schematic/map arose out of the experiences had and the lessons learned as described above that outlines a more straightforward and streamlined pathway through this regulatory and ethical process that will doubtless prove to be of great assistance to novice researchers in the future who are unfamiliar with these systems.

3.6.4.7 Conclusion.

Clinical studies are necessary to derive new empirical knowledge that will advance understanding of human diseases and develop innovative ways of preventing, detecting and diagnosing them. However clinical research involving human participants comes with risks to participants and the importance of protecting the rights, safety and wellbeing of participants cannot be emphasised strongly enough.

This chapter was concerned with the principles of protecting human participants in clinical research and gaining approval from regulatory, ethical and data protection systems to carry out a clinical study at an investigative site. A brief history of clinical research was presented in which landmark examples of gross abuses of human rights in terms of clinical research were highlighted, and how they spurred the development of the regulatory frameworks that are used today to guarantee that research participants are protected to the greatest degree. Loss of access to an investigative site that presented a serious roadblock to the ability of this project to complete its aims was described followed by an account of the time consuming and challenging experience of working with these systems to gain access to an alternative site. Finally, a novel research outcome arising from this experience in the form of a guidance document for engaging with these systems was introduced.

The reader will next be presented a description of this new and useful research outcome in full.

3.6.5 A guidance document for investigative site access

3.6.5.1 Introduction

The guidance that will be described below was developed by this author as a result of experiences had and lessons learned while navigating the regulatory, ethical and regulatory systems that grant approval for clinical research to proceed at an investigative site. It is novel and no such guidance has previously been produced.

3.6.5.2 The guidance document

The following is a draft of the guidance document described in sub-sub section 3.6.5.1.

Guidance for navigating the University of Galway ethical, regulatory and data protection systems that provide approved access to an investigative site for an observational clinical study located at another institute.

This guidance will serve to direct an investigator based at the University of Galway through the necessary regulatory, ethical and data protection systems and obtain the approvals required to allow a non-regulated observational study to be carried out at an investigative site other than the University, in this case, St. James's Hospital Diabetes Day Centre, Dublin.

The guidance will outline the offices at each institution with whom the investigator must engage, and it will list the documentation required by each office. A schematic and instruction sheet are also provided which illustrate how these offices interact with each other and indicate directions for the investigator to follow to ensure that the process is completed in as timely a fashion as possible.

University of Galway research-related offices and systems.

Trials accelerator

The Trials Accelerator is part of the Institute for Clinical Trials at the University of Galway, and it was created to provide support during the initial phases of clinical trial development.^[1] Trials Accelerator personnel will connect a researcher with Institute for Clinical Trials experts who can assist with clinical trial planning, methodological design, drafting protocols, ethics committee applications and navigating regulatory pathways.^[1] Trials Accelerator personnel will

also review inter-institutional documentation such as Data Sharing or Material Transfer agreements and arrange for signatures from appropriate University of Galway personnel.

The Data Protection Office (DPO).

The Data Protection Office assists its organisation (in this case the University of Galway) with all protection issues in relation to personal data. ^[2] The University of Galway DPO advises the institution and its employees of their obligations as they pertain to data protection law. It monitors organisational compliance with data protection legislation and assists with completing data protection impact assessments (DPIAs) which are a requirement for certain research projects. It also acts as the organisation's point of contact for the Data Protection Commission (DPC) and for individuals with concerns regarding processing of their personal data and their data protection rights. ^[2]

The University/employees of the University and not the DPO are responsible for compliance with data protection requirements. ^[2]

Required documentation

- A completed University of Galway Data Protection Impact Assessment (DPIA) Screening Checklist.
- A completed University of Galway Data Protection Impact Assessment (DPIA) Template (current version V2.2).
- A study protocol.
- Participant Information Leaflet(s) (PIL)
- Informed consent form(s) (ICF)
- Data Sharing and Material Transfer Agreements between institutions may be requested for review.

The Clinical Research and Development Office (CRDO)

The Clinical Research and Development Office is staffed by clinical research management personnel from the University of Galway and Saolta University Healthcare Group (SUHG). ^[3] Its primary objective is to 'provide governance for clinical research involving patients, their data or samples, the staff and/or infrastructure of Saolta Group and University of Galway and any co-governed clinical research units, including the Health Research Board Clinical Research Facility Galway (HRB-CRFG), and of regulated and non-regulated trials sponsored by the University of Galway.' ^[3] This office serves to provide institutional oversight and approval for

clinical research, to support researchers and research activity in both institutions, and to facilitate promotion and dissemination of research outputs. It offers a system of clinical research registration, risk assessment and approval, a quality management function, a financial management system for clinical research activities and a process for contract management. ^[3]

Required documentation

- A draft Clinical Trial Agreement/Contract (CTA).
- A Study protocol.
- Participant Information Leaflet(s) PIL.
- Informed Consent Form(s) ICF.
- A certificate/letter of indemnity from the University of Galway Insurance Services Office.
- REC approval letters.
- A completed University of Galway Data protection Impact Assessment (DPIA).
- A sponsor/HSE DPO opinion letter (in this case it will come from the University of Galway DPO).
- A study budget if applicable.

The Insurance Services Office.

The Insurance services office manages the University Insurance programme. ^[4] It is responsible for managing and handling insurance claims and it is also responsible for providing support and advice on insurance matters. ^[4] The office arranges cover for legal liabilities including employer's liability and public liability. ^[4]

Required documentation:

- The Office may request a copy of a completed REC submission package.

Institutional Research Information System (IRIS)

The Institutional Research Information System is a web-based system for university researchers and academic staff that allows them to develop and maintain an online profiles/curriculum vitae and exhibit their research expertise and achievements to a global audience. ^[5]

The CRDO requires that studies sponsored by the University be registered on the principal investigator's IRIS account as part of their clinical trial review process.

Required documentation:

- ICH GCP E6 [R2] training certificates for the University of Galway Principal Investigator and student/investigator co-applicant.
- A study protocol
- Participant Information Leaflet(s) PIL
- Informed Consent Form(s) ICF
- Curriculum vitae for all listed applicants

St James's Hospital research-related offices

Investigative site.

At St. James's Hospital, the investigator will first need to engage with the proposed clinical trial site (in this case the Diabetes Day centre) and gain provisional approval to carry out the study there.

St. James Hospital/Tallaght University Hospital Joint Research Ethics Committee (SJH/TUH JREC).

The St. James's Hospital/Tallaght University Hospital Joint Research Ethics Committee is approved to provide ethical review of research studies in which patients and staff of those hospitals and/or healthy volunteers are either direct participants in a clinical trial or observational study, or their medical records/biological samples are used. ^[6]

Required documentation:

- Letters of approval from other RECs (multi-site studies).
- A study protocol.
- Participant Information leaflet(s) (PIL).
- Informed consent form(s) (ICF).
- Case Report Form(s) (CRF).
- A signed Principal Investigator's curriculum vitae.
- A sample letter for care providers informing them of their patients' participation in the study.
- A certificate/letter of indemnity from the University of Galway Insurance Services Office.
- A dataflow diagram illustrating how data is processed throughout the lifecycle of the study from collection to deletion.

- Additional documentation for example a study master list linking participants to their study number and a copy of the University of Texas staging system for assessing DFU.

St. James's Hospital Research and Innovation (R&I) office.

The St. James's Hospital Research and Innovation Office aims to promote research and build a strong research culture in the institution. ^[7] It has oversight of all hospital-based research activity and serves to protect the wellbeing and rights of staff and patients and to safeguard the institute's resources. ^[7] The office assists researchers with various elements of the research process including ethics application preparation, developing participant information leaflets and data protection. ^[7]

An application to SJH/TUH JREC will not be reviewed unless it has been first approved and endorsed by the R&I office.

Required documentation:

- Letters of approval from other RECs.
- Participant Information Leaflet(s) PIL.
- Informed Consent Form(s) ICFs
- A dataflow diagram illustrating how data is processed throughout the lifecycle of the study from collection to deletion.
- Case Report Form(s) (CRF)
- A Data Sharing Agreement (DSA) and/or a Joint controller Agreement (JCA)
- A Material Transfer Agreement (MTA).
- A Clinical Trial Agreement (CTA) if appropriate.
- A signed non-disclosure agreement (NDA) for non SJH employees who may present at the investigative site at any time
- An invitation for vetting by An Garda Síochána form for non SJH employees who may present at the investigative site at any time.

Recommendations for navigating the regulatory/ethical/data protection systems.

For any post-graduate student/investigator wishing to gain approved access to an investigative site the following steps as illustrated in the site access schematic diagram (Figure 1) should be followed.

Although the processes are presented as sequential steps, they are not intended to be followed in such a manner, and many may be ongoing simultaneously.

Before starting the application process, the student/investigator should get Good Clinical Practice (GCP) and General Data Protection Regulation (GDPR) training from the University of Galway. The CRDO recommends completing the ICH Good Clinical Practice E6 (R2) training course. A link to the course is available on [this web page](#). GDPR training courses can be accessed on [this web page](#). A live online training course is available three times per year and there is also a self-paced course available year-round on Canvas.

1. The first step should be to contact the [Trials Accelerator](#) at the University of Galway Institute for Clinical Trials. Personnel at the accelerator can help with initial stage of trial planning such as developing a study protocol and drafting participant information leaflets (PIL) and Informed consent forms (ICF). The trials accelerator can also locate principal investigators with relevant experience and expertise and provide expertise in regard to navigating ethics submissions and regulatory pathways.^[1]
2. Once a protocol has been developed and PIL/ICFs have been drafted, the student/investigator should make initial contact with the proposed investigative site to gain approval from site personnel to carry out the study there. A site-based Principal Investigator (a consultant employed at the site) will be required.
3. The Data Protection Impact Assessment (DPIA) is central to the approval process. Data Sharing and Material Transfer Agreements will not be signed by appropriate University of Galway personnel until the DPIA has been completed and it is also necessary to complete study registration with the CRDO. The DPIA is a lengthy process and as soon as drafts of the study protocol, PIL and ICF are prepared, contact with the DPO should be initiated.
 - a. A DPIA submission form checklist needs to be completed.
 - b. If the answer to any of the questions on the checklist is ‘yes’, then a Data Protection Impact Assessment will need to be completed by the student/investigator and submitted to the DPO via the Research DPIA portal.
 - c. The DPIA submission form checklist, DPIA template and submission portal are all accessible on the [DPO web page](#).
 - d. The DPO will review the documentation and make recommendations.
 - e. DPO consultants will also ask for drafts of the study protocol, PIL and ICF.

- f. There may be multiple consultations with the office and multiple iterations of submitted documentation until the DPO is prepared to declare the DPIA as finalised.
 - g. Once finalised the DPO will sign off on the DPIA and issue a sponsor DPO opinion letter.
- 4. The student/investigator will need to register the study with the CRDO.
 - a. The study will first have to be registered on the IRIS website.
 - b. The profile page of a study Principal investigator based at the University of Galway will be required and the study must be registered on the 'Grants' page of that profile.
 - c. The student/investigator can gain proxy access to the Principal Investigator's profile to complete this step with the assistance of personnel at the CRDO and University Information Solutions and Services (ISS).
 - d. The student/investigator will next need to complete the online CRDO contract review application form and upload all required documentation (see the documentation required list at the end of the CRDO description above).
- 5. The student/investigator should contact the University of Galway Insurance Services office and confirm that the study is covered under the University Life Science Practice Insurance Policy.
 - a. The office may request a copy of an ethics submission package to facilitate this confirmation.
 - b. A copy of the confirmation of insurance letter can be obtained online.
- 6. The student/investigator will also need to submit an application to have their study reviewed by the SJH/TUH JREC.
 - a. The submission must be made via an online Ethics Review Manager platform.
 - b. The form is quite lengthy and complex. Caution needs to be taken when completing this form as errors will be queried and this will ultimately cause delays in the submission process.
 - c. Required documentation should be uploaded (See 'documentation required' for SJH/TUH JREC above).
 - d. Only University of Galway DPO approved versions of documentation such as PILS, ICFs and the study protocol should be uploaded.

- e. The student/applicant and investigative site principal investigator must sign the form once it is completed. There is a 'request signature' option which sends an email request to the investigative site principal investigator.
 - f. The SJH/TUH JREC does not have submission deadlines. The full REC meets ten times a year and six to ten applications are reviewed at each meeting so it is difficult to predict when a study will undergo review.
 - g. In addition, a submission to this REC will not be accepted for review until a St. James's Hospital Research and Innovation SJH (R&I) form has been completed. This requirement appears at the end of the REC application form.
7. Once the student/investigator completes and signs the SJH/TUH JREC application form and requests the investigative site Principal Investigators signature, the St. James' Hospital Research and Innovation application form must be completed.
- a. Instructions for gaining access to the SJH R&I form appear on the 'SJH Research and Innovation Disclaimer' page which appears at the end of the SJH/TUH JREC application form just before that form's signature page.
 - b. This form is not as lengthy as the ethics application form, nevertheless, it should be completed with caution as errors will contribute to delays in the approval process.
 - c. Required documentation should be uploaded (See 'required documentation' at the end of the St. James's Hospital Research and Innovation (R&I) office section above).
 - d. Only University of Galway DPO approved versions of documentation such as PILS, ICFs and the study protocol should be uploaded.
 - e. Many of the sections in this form overlap with the SJH/TUH JREC application form but these will be prepopulated.
8. In terms of inter-institutional documentation, the lead of SJH R&I office should be followed.
- a. Form templates (Garda vetting, NDA, MTA and DSA) are available on the SJH/TUH JREC Ethics Review Manager platform.
 - b. Click on the 'templates' option in the 'Help' dropdown menu located at the top of the platform page and download the required forms.
 - c. Data Sharing and Material Transfer forms should be edited where highlighted by the student/Investigator.
 - d. Edited forms should be sent to the Trials Accelerator for review.

- e. Trials Accelerator personnel will arrange for the forms to be signed by appropriate University personnel and return them to the student/investigator.
 - f. The student/investigator should then upload the reviewed and signed forms to the St. James R&I application form.
 - g. The forms cannot be signed at the University of Galway until a study DPIA has been finalised.
9. Once all relevant documentation has been uploaded to the SJH JREC and R&I forms, the review process can be completed. Queries to submitted information will likely present and these will be communicated to the student/investigator applicant via email. Queries should be addressed promptly to ensure timely and efficient completion of the review process.

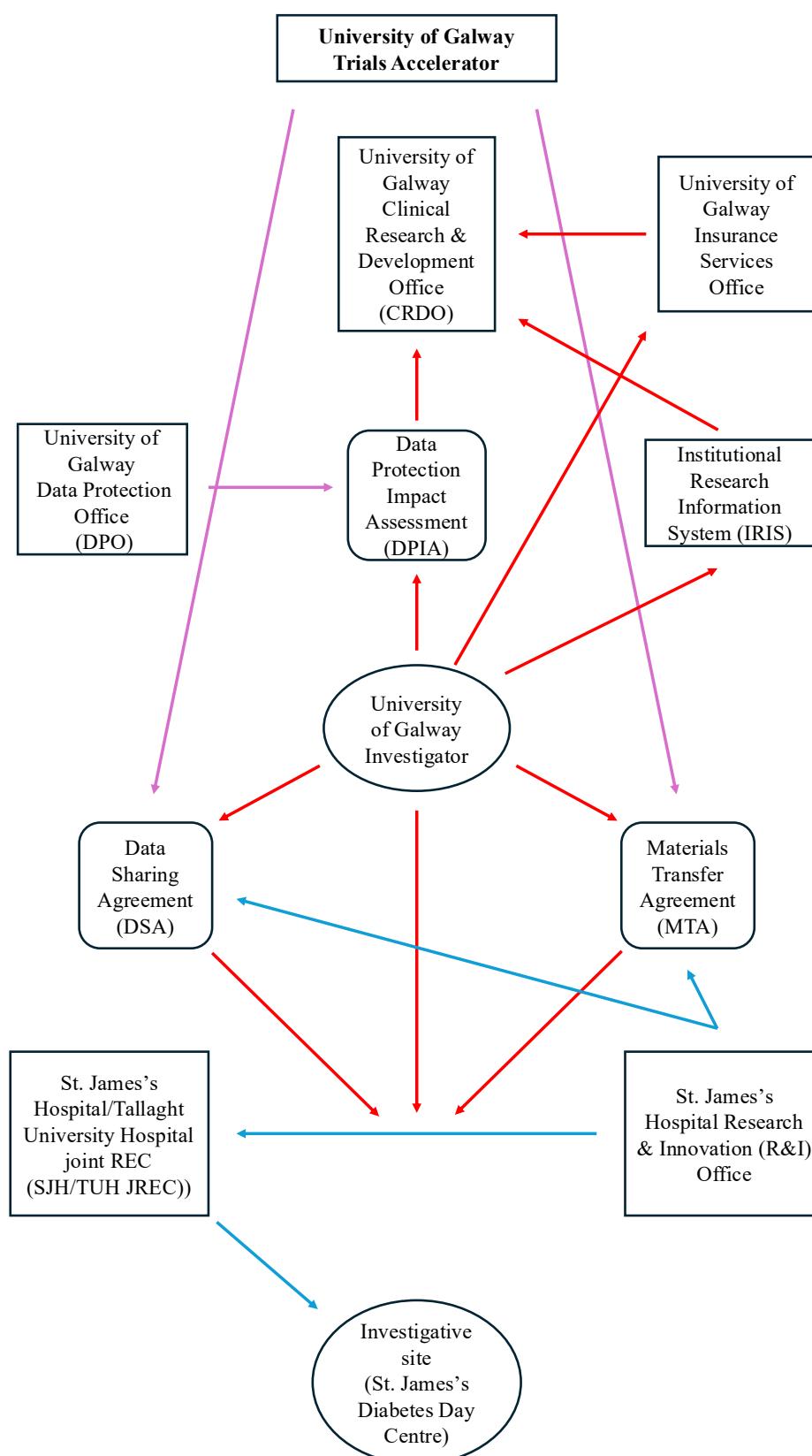


Figure 1. Schematic representation of the regulatory, ethical and data protection pathways that must be navigated by an investigator to gain approved access to an investigative site. Red arrows indicate actions to be completed by the investigator; lilac arrows represent those to be

completed by University of Galway regulatory and data protection offices and blue arrows represent actions to be completed by St. James' Hospital regulatory/ethics offices.

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3.7 Thesis phase 5: A fluorescence staining procedure

3.7.1 Introduction

In this phase, the methodology and preliminary findings of a means of highlighting morphological features of bacterial biofilms using a series of fluorescent dyes in conjunction with CLSM will be presented. The methodology was developed with the assistance of colleagues in Microbiology, School of Biological and Chemical Sciences, University of Galway, and it was devised to act as a substitute for PNA FISH, the pre-prepared kits for which are no longer commercially available.

3.7.2 Fluorescence staining procedure manuscript

The following is a draft manuscript for the imaging procedure referred to in subsection 3.7.1

Fluorescence staining to identify cell aggregate and extracellular matrix components of bacterial biofilms. A pilot *in vitro* study.

Introduction/Background

In nature, bacteria can exist as a planktonic phenotype presenting as single, free-floating cells or as a biofilm phenotype presenting as clusters or aggregates of bacterial cells, with this phenotype representing an estimated 80 to 99.9% of the total microbial biomass on earth. ^[1-3]

These aggregates form communities surrounded by a self-secreted extracellular polymeric substance (EPS) or extracellular matrix (ECM) composed of polysaccharides, proteins, glycolipids, DNA and water that are structurally stable and more than capable of surviving and thriving in hostile environments. ^[2, 4, 5] The communities are complex and highly organised, they are capable of cell-to-cell communication, they demonstrate spatial heterogeneity in terms of metabolic activity and the ECM increases the integrity and strength of the biofilm, protecting the community within from environmental stress, allowing intercommunication through chemical and physical signals and facilitating surface attachment. ^[1, 4, 6-10]

Earlier conceptualisations of biofilms stressed irreversible attachment to a surface, but subsequent research revealed that this is not always the case, and in regard to chronic infections, biofilms are now categorised into surface and non-surface attached subtypes. ^[1, 11, 12] Biofilms are prevalent in chronic wounds and within this unique environment they have been described by the International Wound Infection Institute (IWII) as ‘aggregate microorganisms that have

unique characteristics and enhanced tolerance to treatment and the host defences. Wound biofilms are associated with impaired wound healing and signs and symptoms of chronic inflammation'.^[13-16] It has been proposed that they enter a parasitic relationship with the host, taking control of its inflammatory response within the wound which results in an ongoing state of hyper-inflammation that is destructive to the host but provides a constant supply of nutrients to the biofilm.^[3, 17]

Biofilms can be difficult to diagnose; standard microbial culturing can underestimate the degree of bacterial colonisation and number of species in a wound, clinical signs and symptoms are often used but are largely unvalidated and while advanced microscopy can also be used, it is not readily available at the bedside.^[18] According to the European Society for Clinical Microbiology and Infectious Disease (ESCMID), tissue samples taken from a biopsy are the most reliable means of determining biofilm presence in chronic wounds.^[19] Furthermore, they recommend that biopsies should be examined with techniques such as scanning electron microscopy (SEM) and confocal laser scanning microscopy (CLSM).

With conventional widefield optical epifluorescence, features in the objective focal plane can become blurred and obscured by secondary fluorescence from the specimen, and fluorescent emissions from both above and below the focal plane in thicker samples further reduces resolution of the final image.^[20, 21] CLSM overcomes these problems by using a laser 'specific to the excitation maxima of the dye' which optimises the signal to background noise ratio in addition to a system of pinhole apertures which block light rays reflected from outside the focal plane.^[21, 22]

Fluorescent *in situ* hybridization uses DNA or RNA sequence probes labelled with fluorescent dyes to identify DNA sequences in their natural locations within a chromosome.^[23] The technique is commonly used for identifying and locating bacteria within samples and a further development has been the introduction of synthetic DNA analogues or peptide nucleic acids (PNA) which make FISH procedures more efficient and easier to execute.^[24]

PNA FISH in conjunction with CLSM has been used in chronic wound research to visualise and characterise biofilms, usually in conjunction with techniques such as SEM and DNA sequencing. Malone et al. used PNA FISH with CLSM to confirm presence or absence of biofilm in a study that aimed to determine the effect of cadexomer iodine on microbial load in non-healing diabetic foot ulcers.^[25] Fazli et al. and Kirketerp-Møller et al. used it to examine the spatial distribution and structural organisation of biofilms in venous leg ulcers and in other non-specified chronic wounds.^[26, 27] Johani et al, used the technique to confirm presence or

absence of biofilms in diabetic foot ulcers in a characterisation study and Lichtenberg et al. used PNA FISH with CLSM to characterise the distribution of bacteria in lower leg wounds. [28, 29] Of these five studies, four reported using PNA FISH probes and kits supplied by AdvanDX Inc., Woburn, MA. [26-29]

However, these kits are no longer commercially available, and so we decided to investigate an alternative means of staining biofilms and visualising them with CLSM.

In this study, Methicillin resistant *Staphylococcus aureus* (MRSA) and Methicillin susceptible *Staphylococcus aureus* (MSSA) biofilms grown in the laboratory and stained with a series of fluorescent dyes were imaged with CLSM. This is a preliminary step in a process of optimisation that we anticipate will lead to an effective method alternative to PNA FISH with CLSM for identifying biofilm communities in *ex vivo* tissue sections of biopsies taken from consenting patients with diabetic foot ulceration.

Methods

Growth of MRSA and MSSA biofilms [30]

Methicillin resistant *Staphylococcus aureus* (MRSA) (isolate identifier P-11, taken from a diabetic foot ulcer swab) which produces a primarily protein-adhesin type biofilm and Methicillin susceptible *Staphylococcus aureus* (MSSA) (isolate identifier P-17, taken from a diabetic foot ulcer deep swab) which produces a primarily polysaccharide type biofilm were revived from glycerol stocks by streaking onto Tryptone soya agar (TSA) plates and grown overnight at 37°C.

A single colony from each revived isolate was then selected from the plates, inoculated into 5ml Brain Heart Infusion (BHI) broth and incubated overnight at 37°C with shaking at 250rpm. For biofilm formation, overnight cultures were inoculated into BHI broth, – BHI supplemented with 1% glucose and BHI supplemented with 4% NaCl at a starting OD₆₀₀ of 0.02.

BHI broth supplemented with 1% glucose supports growth of protein-adhesin type biofilms (MRSA) whereas BHI broth supplemented with 4% NaCl supports growth of polysaccharide type biofilms (MSSA).

The biofilms were grown for 24 hours at 37°C in 200µl cultures in the wells of two 8-Well glass Chamber Slides™ (Nunc ® Lab-Tek®, Rochester, NY).

After 24h, the culture medium was discarded and the biofilms in each well were washed twice with 125µl of Phosphate Buffered Saline (PBS) before the biofilms were stained as described below.

Fluorescent dye staining

Five fluorescent dyes were used in this study. Bacterial cell DNA was stained with either 2µg/ml 4',6-diamidino-2-phenylindole (DAPI) (Thermo Scientific™) or 2µg/ml SYTO-9 (ThermoFisher Scientific) green fluorescent nuclear and chrome counterstain.

Biofilm polysaccharide-type ECM was stained with either 5µg/ml Wheat Germ Agglutinin (WGA) (Invitrogen™) or 1:2 Calcofluor White Stain (Sigma-Aldrich): PBS while 1:2 Film Tracer™ SYPRO™ Ruby biofilm matrix stain (Invitrogen™): PBS was used to stain biofilm protein-type ECM.

All dyes were made up in sterile water.

Two hundred microlitres of staining solution was added to the biofilm wells according to the schematic (see Fig 1) and incubated for 30 minutes in the dark.

Thereafter the biofilms were washed twice with 125µl of PBS before the well inserts were removed, and the slides were dried for 30 minutes at 37°C.

Finally, coverslips were put in place on the slides and affixed with nail polish.

Plate 1

MRSA	DAPI	RUBY	DAPI + Ruby	N/A
MSSA	DAPI	WGA	DAPI + WGA	N/A

Plate 2

MRSA	SYTO 9	Ruby	Calcofluor	SYTO-9/ RUBY/Calcofluor
MSSA	SYTO 9	Ruby	Calcofluor	SYTO-9/ RUBY/Calcofluor

Figure 1. Staining and isolate arrangement schematic for 8-Well glass Chamber Slides™ (Nunc® Lab-Tek®, Rochester, NY).

MRSA = Methicillin resistant *Staphylococcus aureus*, MSSA = Methicillin susceptible *Staphylococcus aureus*, DAPI = 4',6-diamidino-2-phenylindole, Ruby = Film Tracer™ SYPRO™ Ruby biofilm matrix stain (Invitrogen™), WGA = Wheat Germ Agglutinin (Invitrogen™), SYTO 9 = SYTO 9 green fluorescent nuclear and chromosome counterstain (ThermoFisher Scientific), Calcofluor = Calcofluor White Stain (Sigma-Aldrich).

Confocal Laser Scanning Microscopy (CLSM)

Stained biofilms were imaged at X1000 magnification using an Olympus LS FLUOVIEW FV3000 confocal laser scanning microscope.^[31]

Fluorescent images were obtained using excitation wavelengths of 405nm, 488nm and 561nm for DAPI, SYTO-9 and Ruby respectively (Calcofluor white is similar to DAPI in terms of excitation as is WGA to Ruby). Filters were set so that fluorescence emission was recorded within the ranges of 430-470nm for blue, 500-540 for green and 570-670nm for red (Table 1).

	Excitation (nm)	Emission (nm)	Laser power (%)
DAPI	405	430 – 470	1.5
SYTO-9	488	500 – 540	10
Ruby	561	570 - 670	4

Table 1. Excitation and emission wavelengths used for DAPI, SYTO-9 and Ruby fluorescent dyes.

DAPI = 4',6-diamidino-2-phenylindole, SYTO 9 = SYTO 9 green fluorescent nuclear and chromosome counterstain (ThermoFisher Scientific), Ruby = Film Tracer™ SYPRO™ Ruby biofilm matrix stain (Invitrogen™).

CLSM Images were uploaded to ImageJ^[32] for processing and presentation.

Results

In figure 2 below, images of DAPI (nucleic acids) + Ruby (ECM protein) and composite DAPI + Ruby stained P-11 isolate MRSA biofilm can be seen. Each row represents a different layer throughout the structure of the biofilm captured by CLSM.

The DAPI + Ruby composite images show dense bacterial cell aggregation with DAPI stained nucleic acids (blue) interspersed with Ruby stained protein-type ECM (red). With respect to the individual DAPI and Ruby images, the dense and less dense (bright or less bright) concentrations of ECM protein appear to correspond spatially with the dense and less dense concentrations of cell aggregations. Void areas (black) where presumably few or no cells exist also correspond spatially across all images in each layer. Good resolution images were captured across each layer of the biofilm.

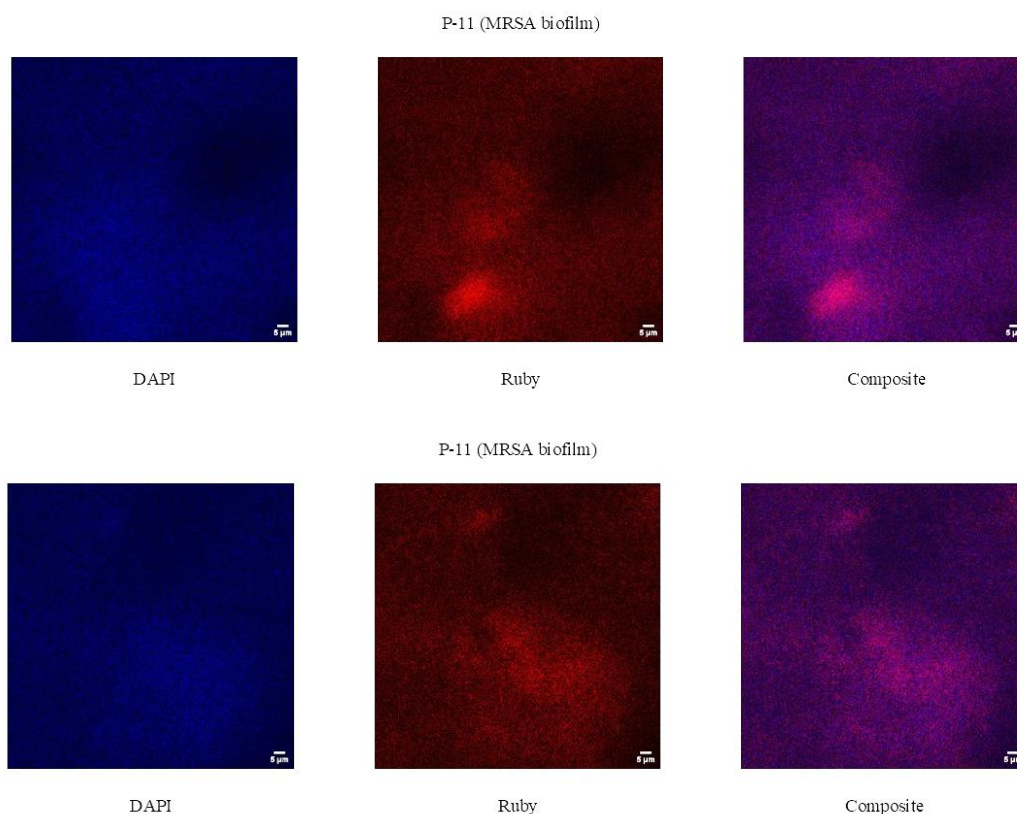


Figure 2. Images showing P-11 isolate MRSA biofilm stained with DAPI alone for nucleic acids (left side), Ruby alone for ECM protein (centre) and composite DAPI + Ruby (right side). Each row represents a different layer throughout the structure of the biofilm captured by CLSM. MRSA = Methicillin resistant *Staphylococcus aureus*, DAPI = 4',6-diamidino-2-phenylindole, Ruby = Film Tracer™ SYPRO™ Ruby biofilm matrix stain (Invitrogen™), ECM = extracellular matrix, CLSM = confocal laser scanning microscopy.

Figure 3 shows images of Calcofluor white (ECM polysaccharide) + SYTO-9 (nucleic acids) + Ruby (ECM protein) stained P-11 isolate MRSA biofilm. Each row represents a different layer throughout the structure of the biofilm captured by CLSM.

These images also demonstrate densely packed bacterial cells with SYTO-9 stained nucleic acids (green).

In the first two layers, the dense and less dense (bright and less bright) concentrations of both protein (red) and polysaccharide-type (blue) ECM correspond spatially with the dense and less dense concentrations of cell aggregations. The concentrations and the distributions of protein and polysaccharide-type ECM (individual Ruby and Calcofluor images) in the first two layers appear to be similar. However, in the third layer, they oppose each other; more dense concentrations of protein-type ECM correspond to less dense concentrations of polysaccharide-

type ECM and the more dense concentrations of protein-type ECM correspond to more dense concentration areas of cell aggregations.

The composite images of the first two layers are primarily green with minimal blue and red emphasising the cell aggregations, but the composite image of the third layer is largely blue and red reflecting a more heterogenous distribution of polysaccharide and protein-type ECM across the cell aggregations.

Void areas also correspond spatially across the images of the first two layers but not the third. In this series the quality of imaging is inconsistent across the captured layers of the biofilm with blurring and staining artifacts evident in the second and third layers respectively.

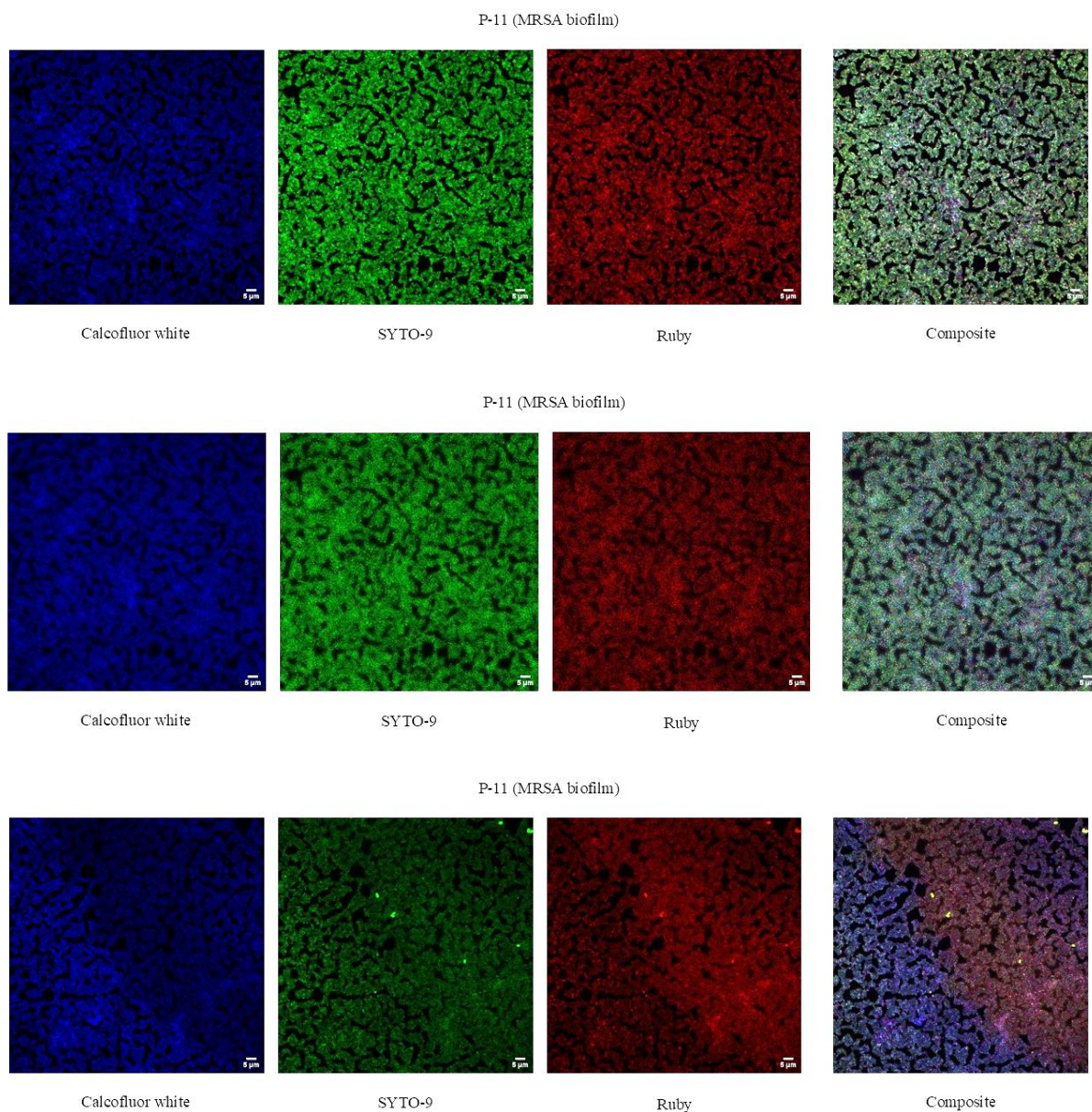
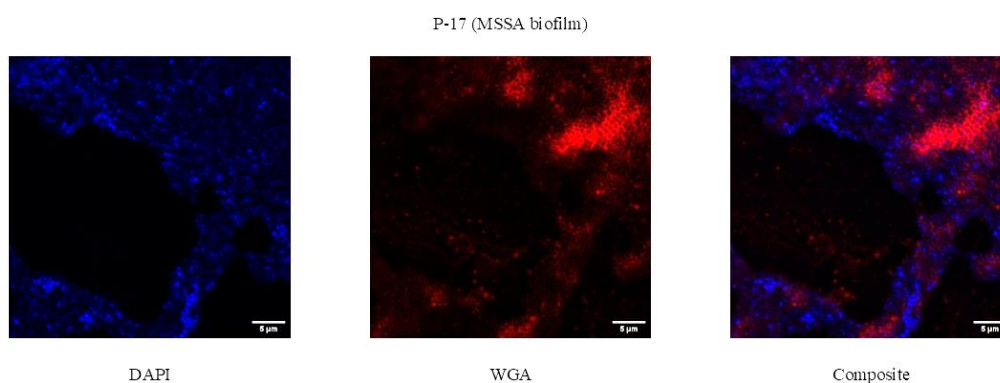


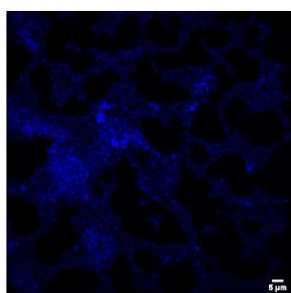
Figure 3. Images showing P-11 isolate MRSA biofilm stained with Calcofluor alone for ECM polysaccharide (far left), SYTO-9 alone for nucleic acids (centre left), Ruby alone for ECM protein (centre right) and composite Calcofluor + SYTO-9 + Ruby (far right). Each row represents a different layer captured throughout the structure of the biofilm captured by CLSM. MRSA = Methicillin resistant *Staphylococcus aureus*, Calcofluor = Calcofluor White Stain (Sigma-Aldrich), ECM = extracellular matrix, SYTO 9 = SYTO 9 green fluorescent nuclear and chromosome counterstain (ThermoFisher Scientific), Ruby = Film Tracer™ SYPRO™ Ruby biofilm matrix stain (Invitrogen™), CLSM = confocal laser scanning microscopy.

Figure 4 shows images of DAPI (nucleic acids) + WGA (ECM polysaccharide) stained P-17 isolate MSSA biofilm. Each row represents a different layer throughout the structure of the biofilm captured by CLSM.

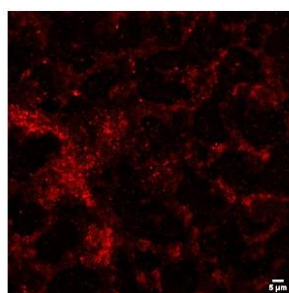
The DAPI + WGA composite images show bacterial cell aggregations with DAPI stained nucleic acids (blue) associated with WGA stained polysaccharide-type ECM (red). With respect to the individual DAPI and WGA images, dense and less dense (bright or less bright) concentrations of ECM protein spatially correspond most closely with dense and less dense cell aggregations in layers two to four and less so in layers one and five. Void (black) areas appear to correspond across all images. The quality of imaging is consistently good with the exception of layer six in which the images are uninterpretable.



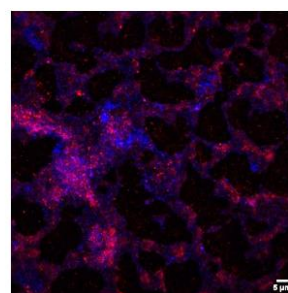
P-17 (MSSA biofilm)



DAPI

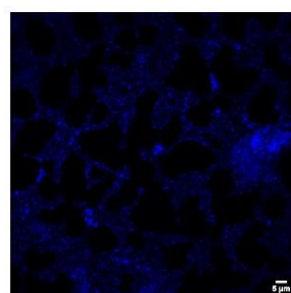


WGA

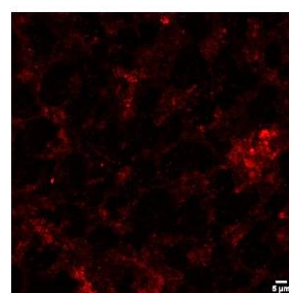


Composite

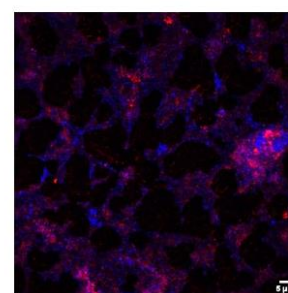
P-17 (MSSA biofilm)



DAPI

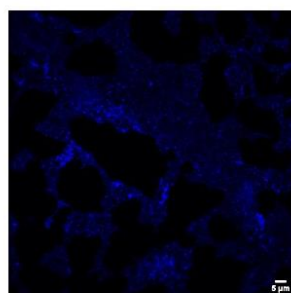


WGA

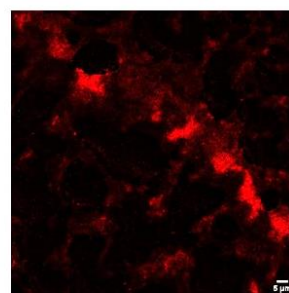


Composite

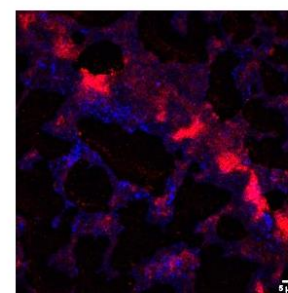
P-17 (MSSA biofilm)



DAPI

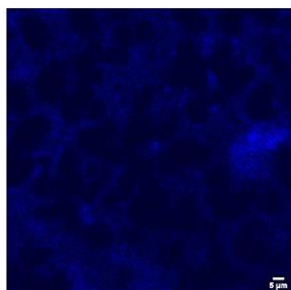


WGA

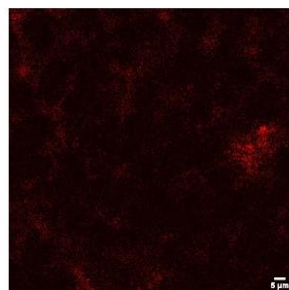


Composite

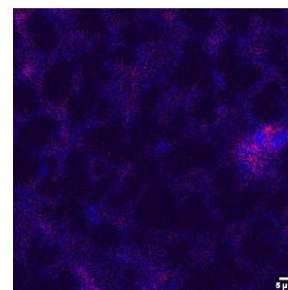
P-17 (MSSA biofilm)



DAPI



WGA



Composite

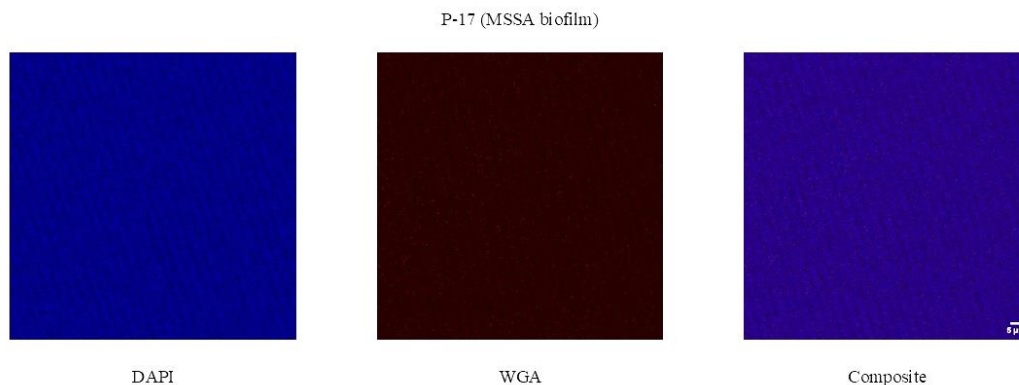


Figure 4. Images showing P-17 isolate MSSA biofilm stained with DAPI alone for nucleic acids (left side), WGA alone for ECM polysaccharide (centre) and composite DAPI + WGA (right side). Each row represents a different layer captured throughout the structure of the biofilm by CLSM.

MSSA = Methicillin susceptible *Staphylococcus aureus*, DAPI = 4',6-diamidino-2-phenylindole, WGA = Wheat Germ Agglutinin (InvitrogenTM), ECM = Extracellular matrix, CLSM = confocal laser scanning microscopy.

Figure 5 shows images of Calcofluor white (ECM polysaccharide) + SYTO-9 (nucleic acids) + Ruby (ECM protein) stained P-17 isolate MSSA biofilm. Each row represents a different layer throughout the structure of the biofilm captured by CLSM.

As with the P-11 isolate MRSA strain biofilm, densely packed bacterial cells with SYTO-9-stained nucleic acids (green) can be seen.

In the first two layers there appears to be more Ruby stained protein-type ECM (red) than Calcofluor stained polysaccharide-type ECM (blue), with the protein-type ECM covering more surface area. In terms of dense and less dense (bright or less bright areas) concentrations of cell aggregations and each ECM type, the protein-type ECM corresponds spatially with the biofilm cell aggregations while the polysaccharide-type ECM appears to occupy spaces where the density of cell aggregations is lower. Void (black) areas match across the protein-type ECM and cell aggregation images in these layers.

In terms of quality, the third layer is dim, and the images are less clear than in the first and second layers making it difficult to pick out individual features.

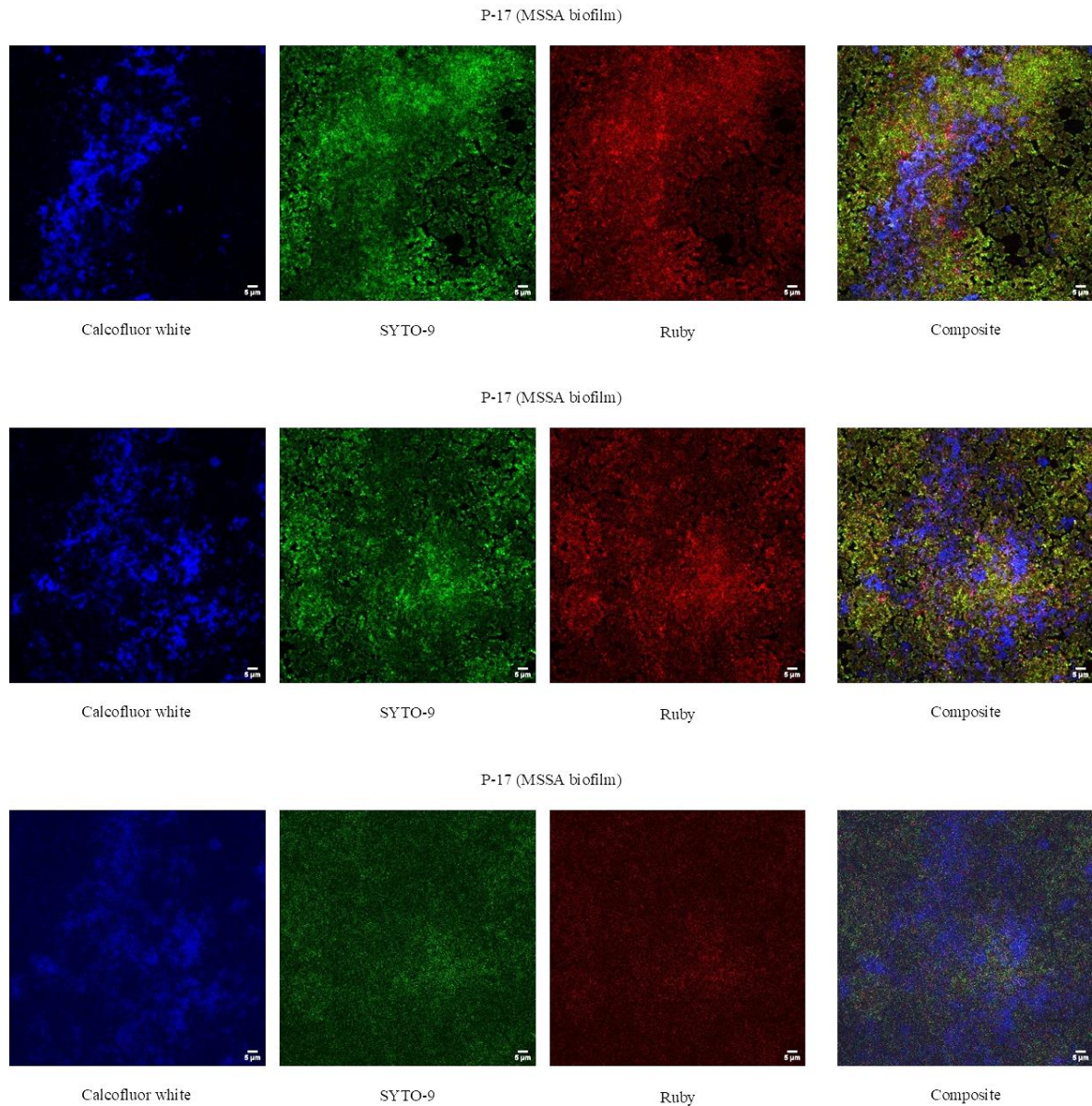


Figure 5. Images showing P-17 isolate MSSA biofilm stained with Calcofluor alone for ECM polysaccharide (far left), SYTO-9 alone for nucleic acids (centre left), Ruby alone for ECM protein (centre right) and composite Calcofluor + SYTO-9 + Ruby (far right). Each row represents a different layer captured throughout the structure of the biofilm captured by CLSM. MSSA = Methicillin susceptible *Staphylococcus aureus*, Calcofluor = Calcofluor White Stain (Sigma-Aldrich), ECM = extracellular matrix, SYTO 9 = SYTO 9 green fluorescent nuclear and chromosome counterstain (ThermoFisher Scientific), Ruby = Film Tracer™ SYPRO™ Ruby biofilm matrix stain (Invitrogen™), CLSM = confocal laser scanning microscopy.

Discussion

We wish to develop an optimised means of identifying biofilm communities in tissue samples taken from patients with diabetic foot ulceration that can be used as an alternative to PNA FISH staining in conjunction with CLSM. In this initial step we stained laboratory grown biofilms of MRSA and MSSA strains from patient isolates with combinations of five different fluorescent stains.

ESCMID guidelines outline a series of features that characterise microbial biofilms.^[19] These include structured microbial cell groupings (mono or polymicrobial), a self-produced surrounding polymer matrix, adherence to or association with natural or artificial surfaces within the host and physiological/biochemical gradients. Of these, previous studies that have incorporated the PNA FISH methodology for biofilm identification purposes have typically chosen cell aggregations, a surrounding polymeric substance and adherence to or association with a host surface as defining features.^[25, 27, 28] In an effort to ensure that our method would be capable of illustrating these similar features, the dyes used were chosen on the basis of their specificity for 1) nucleic acids to highlight bacterial cell aggregations (DAPI and SYTO-9), 2) protein-type ECM (Ruby) and 3) polysaccharide-type ECM (WGA and Calcofluor white).^[33-36]

The double-stain combinations – DAPI with Ruby for protein-type ECM (P-11 isolate MRSA) and DAPI with WGA for polysaccharide-type ECM (P-17 isolate MSSA) both appeared capable of illustrating aggregations of bacterial cells in close association with protein or polysaccharide-type ECM. Similarly, the triple-stain combination of SYTO-9 with Calcofluor and Ruby for polysaccharide and protein-type ECM respectively also seemed capable of illustrating bacterial aggregates and associated protein and polysaccharide-type ECM.

Biofilms produced by the MRSA and MSSA strains had distinct features. The DAPI + Ruby stained P-11 MRSA strain biofilm presented as a dense aggregation of cells with an equally dense concentration of ECM that filled almost the entire image, with few, vaguely defined void spaces. The DAPI + WGA stained P-17 MSSA strain biofilm on the other hand, while showing similar, equally distributed concentrations of cell aggregations and ECM, was more nebulous with larger, more clearly defined void areas. These different biofilm characteristics may reflect differences between MRSA and MSSA biofilm or may be peculiarities of the two strains used in this study. The composite images for the biofilms formed by each strain illustrated both biofilm characteristics well and the presentation patterns remained more or less consistent throughout the captured layers.

The SYTO-9 + Calcofluor + Ruby stained P-11 MRSA biofilms presented as a dense aggregation of cells filling the entire image with clearly defined void areas. Both the protein and polysaccharide-type ECM patterns matched the cell aggregate pattern equally in terms of distribution and density. MRSA produces a predominantly protein-type ECM and so it was unexpected to see equal amounts of protein and polysaccharide-type ECM present in the images. It is not clear why this would be the case - the images represent limited cross-sections throughout the whole biofilm, and it is possible that images from sections in other parts of the biofilm would exhibit a different, more protein-dominant scenario. Indeed, the third layer demonstrates differing distributions of protein and polysaccharide-type ECM compared to the first two. The composite images in two of the layers illustrated a preponderance of cell aggregates but very little ECM, while that from the third layer was more realistic with greater representation of ECM components. This stresses the importance of capturing images from multiple layers throughout the biofilm and also the importance of not viewing composite images alone but rather in the context of their individual component images. We believe that such practices would mitigate the risk of false negative findings in terms of confirming presence of either of the biofilm components in wound biopsies.

The SYTO-9 + Calcofluor + Ruby stained P-17 MSSA strain also presented as a dense aggregation of cells but with less clearly demarcated void areas. The protein-type ECM matched the cell aggregate pattern equally in regard to distribution and density in the first two layers. The arrangement of the polysaccharide-type ECM did not however and there was less of it than there was of protein-type ECM which is not typical for this strain. However, in the third layer, there appears to be more polysaccharide-type ECM, again showing that biofilms are not uniform and that images from one section are not necessarily representative of the whole biofilm. The composite images for this strain clearly highlighted both the cell aggregate and ECM biofilm components.

There are many observations and interpretations that could be made in relation to these images, and while the different presentations are interesting and have provided much information, the aim of this work was to devise a means of identifying cell aggregates and ECM, two characteristics of a biofilm as specified in ESCMID guidelines and we believe that we have achieved this. ^[19]

An alternative to the experiments performed here would have been to source probes and reagents for PNA FISH and attempt to develop that process in our laboratory. However, given our experience with fluorescence microscopy, we decided to use this approach.

Our process was straightforward and was executed in five steps compared to three for PNA FISH. [27, 28] We were able to complete it for two samples in approximately 2.5 – 3 hours compared to 2 – 2.5 hours for PNA FISH, and we envisage that it could be easily scaled up to manage larger numbers of samples within in a similar time frame. [26-28]

PNA FISH labels nucleic acid sequence targets within a cell with fluorescently labelled complementary probes. [37] In terms of biofilm imaging these probes can be species-specific or generic, [25, 38] but regardless, they bind exclusively to nucleic acids within bacterial cells and so this methodology can only highlight cell aggregates. Researchers who have used PNA FISH with CLSM to image biofilms in wounds typically used it for this purpose, but they also incorporated a second methodology such as SEM or a separate staining process to demonstrate the presence of ECM. [25, 27, 28] In a study to optimise FISH and CLSM for the purposes of characterising the spatial organisation of biofilms *in vitro*, Thurnheer et al. used Calcofluor to stain biofilm ECM *after* the FISH procedure was completed. [38] An advantage of our procedure is that it was singlehandedly capable of highlighting both features. However, we achieved this under *in vitro* conditions in which the only cells present were bacterial. In a section of tissue from a wound biopsy the specific nature of PNA probes becomes more advantageous as they will exclusively highlight bacterial cells but not those of the host tissue. We used DAPI stain to highlight bacterial cell DNA and thus illustrate presence of bacterial cell aggregates. DAPI however is non-specific, labelling A-T rich sequences in DNA and in an *ex vivo* scenario this would include the DNA present in the cells of host tissue. [39]

The next step in optimising this method for identifying biofilm components should be to test it under *ex vivo* conditions for example on pig skin. We chose *S. aureus* isolates for this work because it is one of the four most prevalent bacterial species in chronic wounds. [40] We therefore anticipated that its biofilms would have good external validity and generalizability with respect to biofilms to be found in chronic wounds and we think that the next stage should involve similar strains. Punch biopsies would be taken from appropriately inoculated pigskin samples and individual sections taken at different depths of the biopsy. These sections would then be mounted and stained using the procedure outlined here and examined for biofilm with CLSM. A primary concern to be addressed at this next stage would be to see how stained biofilms will present in the context of host tissue. The Calcofluor, WGA and Ruby stains are considered to be specific for biofilm ECM, and we do not anticipate any issues, but DAPI will stain both host and bacterial DNA. However, there is an observable size difference between prokaryotic and eukaryotic cells, and because eukaryotic cell DNA is contained within the nucleus, void areas

should be visible around the DAPI stained nuclei. We expect that biofilm cell aggregates should be identifiable in an *ex vivo* context on the basis of these factors.

Recommendations

We would advise that multiple layers are imaged and that single component images as well as composites be taken into consideration when confirming presence of ECM and cell aggregates. Capturing multiple layers throughout the sample will help to control for variations in biofilm structure and composition as well as variations in image quality, while single component images in a layer can sometimes reveal biofilm components that have become obscured in composite images.

Ultimately we hope to transfer this method for confirming presence of biofilm components to sections of biopsies taken from the foot ulcers of persons with diabetes. Although it has proven capable of identifying two components of bacterial biofilm (whereas PNA FISH only confirms one), we do not see it as a standalone technique and recommend that it be used in conjunction with SEM for cross-validation purposes.

Limitations

The sample size for this work was small and uncontrolled given that the objective was to determine if the staining scheme would be capable of highlighting biofilm components. However, now that we have shown the potential of this method to highlight cell aggregates and ECM components of bacterial biofilm of isolates cultured from the foot ulcers of persons with diabetes in an *in vitro* context, the next *ex vivo* phase in the optimisation of this method using pigskin inoculated with *S. aureus* will incorporate larger number of samples and a more realistic context. This phase will also incorporate control sections taken from uninoculated areas of the pig skin samples.

Conclusion.

Peptide nucleic acid fluorescence *in situ* hybridization in conjunction with confocal laser scanning microscopy is an accepted method for confirming biofilm presence in chronic wounds. The methodology is capable of highlighting the cell aggregation components of biofilms and is commonly used alongside SEM or other staining techniques which confirm presence of ECM. However, PNA FISH kits are no longer commercially available and although it is potentially possible to source kit materials individually and recreate the process, we decided to attempt to identify biofilm components using five different specific fluorescent dyes instead. Initial

trialling showed the process to be remarkably successful and capable of highlighting both cell aggregations and ECM components of *S. aureus* biofilms grown *in vitro* which PNA FISH cannot do alone.

We cautiously anticipate that subsequent investigation of the process in a more challenging *ex vivo* context will show it to be similarly effective.

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3.8 Chapter conclusion

The reader has now arrived at the end of the methods and results chapter in which a published protocol for the scoping review's methodology followed by a published manuscript of its findings were presented (Phase 1). A published protocol for the electronic Delphi study's methodology came next followed by a published manuscript of its findings. (Phase 2). A protocol for a clinical study was then presented (phase 3) that will allow the predictive abilities of the list of clinical signs and symptoms from phase two to be ascertained statistically with a subsequent narrative describing the experience of working through regulatory, ethical and data protection systems to gain approved access to an investigative site for the study. A guidance document for novice researchers was created from this experience to facilitate investigative site access, and this followed the narrative (Phase 4). Finally, the methodology and findings for an imaging process to identify prominent features of bacterial biofilms were exhibited (phase 5). The reader is now directed to the discussion chapter where each of these phases will be critically reviewed.

4 Chapter Four: Thesis discussion and conclusion

4.1 Introduction

In this chapter the reader will first be briefly reminded of the gaps in the knowledge that gave rise to the rationale for this PhD project and its aims and objectives. The findings of each of the methodological phases of this work will be summarised as a whole and then discussed in the context of lessons learned, in the context of the literature and in the context of today's clinical research processes where appropriate.

4.2 Review of thesis rationale/aims

It is generally accepted that biofilms contribute to delayed healing in chronic wounds and treatment strategies have been developed that are thought to be effective at managing them and helping to redirect the wounds onto an optimal healing trajectory (Schultz et al., 2017, Metcalf et al., 2014, Swanson et al., 2022, Goswami et al., 2023, Razdan et al., 2022). However, there are some concerns in terms of the safety and effectiveness of these strategies and also in terms of existing techniques used to confirm biofilm presence in chronic wounds (Razdan et al., 2022, Attinger and Wolcott, 2012, Swanson et al., 2022, Wolcott and Rhoads, 2008, Weigelt et al., 2021, Wu et al., 2019a, Darvishi et al., 2021, Wei et al., 2019, Høiby et al., 2015). These treatment and identification concerns highlight a significant gap in the knowledge such that if biofilms cannot be readily confirmed in a wound, how can it be determined that they are there in the first place, that treatment strategies have effectively removed them and that their removal truly facilitates wound healing. Furthermore, if chronic wound biofilm treatment strategies are continuously administered in the face of these uncertainties, there may be a risk of endangering good antimicrobial stewardship. However, a practical tool that is demonstrably capable of confirming biofilm in chronic wounds that can be easily and conveniently used in a clinical setting might help address these concerns.

Statistically validated predictive tools based on clinical signs and symptoms developed through the CPR approach are commonly used in practice to help confirm the presence of different diseases and conditions, and indeed clinical signs and symptoms are commonly used to indicate presence of biofilm in chronic wounds (Tamariz et al., 2004, Beattie and Nelson, 2006, Ivory

et al., 2025a, Yelland et al., 2010, Stiell, 1996). However very little work has been done to statistically validate them in this biofilm/chronic wound context and so it was proposed that an attempt should be made to create for the first time, a predictive tool comprising validated clinical signs and symptoms that could readily help to confirm biofilm in chronic wounds in a clinical setting (Ivory et al., 2025a). The CPR approach is a lengthy and resource intensive process though (Cowley et al., 2019), and so the aim of this PhD project was to initiate a comprehensive process designed to direct research toward developing a clinically available tool that would have statistically determined abilities to predict presence of biofilm in diabetic foot ulcers. Achieving this aim was contingent on completing a series of five interdependent objectives: to complete a scoping review and collate a complete list of all known signs and symptoms reported in the literature to be indicative of chronic wounds; to complete an electronic Delphi study to allow an international panel of wound care clinicians to refine that list via their experience and clinical expertise; to develop a protocol for a clinical study to assess DFU for the presence of signs and symptoms on the refined list and to take punch biopsies of wound tissue; to create a pathway to guide a researcher through the regulatory systems underpinning clinical research to facilitate timely and approved access to an investigative site to execute the clinical study and to develop an imaging process to identify biofilms in these biopsies .

The results of each of these objectives will next be summarised together as a single unit to impress the cohesive nature of this PhD project on the reader and then they will be discussed individually, but first, the author would like to point out that that methodological protocols for the scoping review and electronic Delphi work have been published, the findings of the scoping review have been published, the findings of the electronic Delphi work have been published and the laboratory process has been written up in a format that will allow it to be submitted for publication. Copies of the manuscripts for each of these objectives were incorporated into the methods and results chapter of this thesis, each with its own discussion section. Meaningful points were raised in these discussion sections, and they will need to be reiterated and brought together in this chapter to bring more cohesiveness to this work as a whole.

4.3 Summary of thesis phase results

The scoping review yielded 830 accounts within its 111 included reports of a clinical sign or symptom thought to be indicative of biofilm in chronic wounds and these were grouped into a

more manageable list of 26 signs/symptom items through an inductive content analysis process (Ivory et al., 2025a).

The electronic Delphi process brought together a panel of 103 wound care clinicians from 22 countries with considerable levels of clinical expertise who distilled those 26 signs and symptoms from the scoping review down to a final list of 11 signs and symptoms which, in their opinion, were most likely to be indicative of biofilm in chronic wounds. This list of 11 items was then incorporated into a methodologically rigorous and ethically approved protocol for a clinical study in which the DFU of a sample of consenting patients can be assessed for the presence of items on this list and biopsies of their wound tissue assessed for biofilm presence in the laboratory. A novel guidance document was created which is capable of helping researchers navigate their way through the ethical, regulatory and data protection systems to gain approved investigative site access. This guidance document was favourably reviewed by members of the Trials Accelerator team at the University of Galway's Institute for Clinical Trials.

Finally, the laboratory imaging process demonstrated itself capable of highlighting bacterial cell aggregates and ECM components of biofilms grown *in vitro*, components that have been used by other researchers in the field to confirm biofilm presence in biopsy tissue (Johani et al., 2017, Malone et al., 2017b, Kirketerp-Moller et al., 2008).

4.3.1 The scoping review

The scoping review phase of this PhD project which has been published (Ivory et al., 2025a), was a large and intensive undertaking, and the assistance of seven colleagues was required to ensure that the evidence selection and data charting process were completed in as timely a manner as possible. Systematic reviews and scoping reviews are ideally carried out *a priori*, meaning that a protocol is prepared in advance which provides a clear and structured process for their conduct with reduced risk of bias (Porritt et al., 2024, Rombey et al., 2019). A carefully designed protocol was prepared for this scoping review and published on the Health Research Board's (HRB) Open Research website, and it helped to keep this extensive review on track and guarantee the rigour of its methodology (Ivory et al., 2021).

This is the first time that a scoping review of this magnitude has been carried out in regard to signs and symptoms of biofilm in chronic wounds. Every account of a sign or symptom in its included articles which were located through a comprehensive literature search (Appendix 1) was diligently recorded, categorized, and summarised into a complete list of 26 succinct items.

This list has been disseminated through publication in The International Wound journal, an internationally recognised wound care journal and presentation at an international conference, making it available for anyone who wishes to carry out research relevant to this topic.

The review could have been terminated after data had been extracted from 50% or possibly even less of the included articles because novel or previously unheard of sign and symptom accounts were failing to appear at this point. However, by seeing the review through to completion, that is charting data from all included articles, researchers can be left in no doubt as to the completeness of the existing sign and symptom list and furthermore, it means that the review is likely definitive and will not need to be updated in the future.

A key observation made during the execution of this scoping review was that signs and symptoms in the literature are largely recommended for use to confirm biofilm presence in chronic wounds on an *ad hoc* basis, that is to say with little or no evidence to confirm their true predictive abilities. This observation has been discussed in the published report of this review (Ivory et al., 2025a), but it is an important point, and it will be reiterated over the following paragraphs because it lends strong credence to the aim of this PhD project.

Visual indicators have their proponents and detractors and this controversy has been described in subsection 2.10.6 of this thesis, and the reason for the acceptance of clinical cues may stem from the persistent and progressive pathology of biofilm infections which is hypothesised to be a result of the host's local inflammatory response (Høiby et al., 2015). Furthermore, features such as persistent infection of more than seven days' duration, signs of infection (tumor, rubor, dolor, loss of function, low-grade fever), failure of antibiotic treatment and recurrence of infection, systemic signs and symptoms of infection that resolve with antibiotic therapy but recur on cessation of treatment are reported to be commonalities across biofilm infections other than in chronic wounds, for example periodontitis, cystic fibrosis lung infections, osteomyelitis, endocarditis and otitis media (Høiby et al., 2015, Hall-Stoodley et al., 2012).

Regardless of the speculations as to why the indicators reported in this scoping review might be taken at face value for the purposes of identifying biofilm in chronic wounds, of the 111 included articles which spanned 22 years and reported visual and clinical cues for biofilm in chronic wound's, only two demonstrated efforts to determine their predictive abilities empirically, and a third study attempting same was published after the scoping review was completed (Johani et al., 2017, Hurlow et al., 2016, Mayer et al., 2024). Using SEM and histology, Hurlow et al. identified biofilm in 75% of debridement samples taken from 11 consenting participants with chronic non-healing wounds that were either partially or

completely covered by a ‘firmly adherent gel-like wound bed film which had reformed under antiseptic wound dressings and sometimes despite guided systemic antibiotics’ (Hurlow et al., 2016). Johani et al. attempted to correlate six clinical observations in wounds (slough, excess exudate, poor tissue quality, signs of pyocyanin, gelatinous wound surface and gelatin reforming quickly) against CLSM and PNA-FISH of biopsies taken from 26 wounds (Johani et al., 2017). Aside from excessive exudate, they reported that the probability of these signs facilitating identification of biofilm in wounds was no better than chance (Johani et al., 2017). Mayer et al. evaluated the abilities of a fluorescence imaging device, a wound blotting technique and clinical signs and symptoms to detect biofilm by comparing them against wound scrapings assessed for biofilm by SEM and PCR microbiology in a sample of 40 patients with chronic wounds (Mayer et al., 2024). They found the sensitivity, specificity and accuracy of the clinical signs and symptoms to be 44%, 40% and 43% respectively (Mayer et al., 2024).

The Hurlow et al. study showed promising results, but it appears to be preliminary work, investigating a single biofilm indicator with a very small sample size, and no diagnostic data in terms of sensitivity, specificity, positive or negative predictive values was presented (Hurlow et al., 2016). The results of the Johani et al. study were less promising and their findings in regard to a gelatin wound surface contradicted those of Hurlow et al. (Hurlow et al., 2016, Johani et al., 2017). The Johani et al. study used a gold standard biopsy, had a larger sample size and used binomial probabilities to calculate predictive abilities of their chosen signs and symptoms. However, this validation exercise was a secondary outcome in their work (Johani et al., 2017). The Mayer et al. study which had the largest sample size demonstrated low (<50%) sensitivity, specificity and accuracy for clinical signs of biofilm (Mayer et al., 2024). It had the largest sample size of the three studies, but the assessment of clinical signs of biofilm was not a primary outcome and it is not clear from the study how the signs’ predictive abilities were assessed, that is, as single entities or as an aggregate/combination of signs (Mayer et al., 2024). Furthermore only one of these studies used biopsy tissue to confirm biofilm presence (Johani et al., 2017), the others used debridement samples or what could be interpreted as a scraping from the wound bed (Mayer et al., 2024, Hurlow et al., 2016). Guidelines for the diagnosis and management of biofilm infections produced by ESCMID advocate biopsy tissues to be the most reliable sample types to assay for biofilm (Høiby et al., 2015). The guidelines consider collecting samples from the wound surface with a swab to be inadequate because of contamination from skin flora, strongly adherent biofilms to epithelial tissue and anaerobes located in the deeper tissues, and one must ask if the same limitations apply to wound scrapings

or debridement samples (Høiby et al., 2015). These guidelines date back to 2014, but an article published in 2023 reported that in the interim opinion of the ESCMID Study Group on Biofilms (ESGB), they do not require revision as of 2022 (Høiby et al., 2023).

The evidence then in terms of biofilm signs and symptoms and their predictive abilities in a chronic wound context is preliminary, limited, heterogenous in terms of methodology and contrary in terms of findings. This observation solidly reinforces the need to engage in a focused, coherent and concerted research effort to take items from this list of supposed indicators and statistically determine their abilities to predict presence of biofilm in chronic wounds.

In summary, this robust scoping review has achieved its objective and provided a list of signs and symptoms reported in the literature to be indicative of chronic wounds that is comprehensive, complete, unlikely to need updating in the future and freely available for use by other wound care researchers. An added bonus of this review was its unexpected ability to add further confirmation to a significant gap in the knowledge, providing further justification for this PhD project.

4.3.2 The electronic Delphi consensus study

The electronic Delphi phase of this PhD project has been published in the International Wound Journal, an internationally recognised, peer reviewed wound journal. It has solid and reliable foundations both in the rigorous scoping review that informed it and in its carefully planned and designed, published methodological protocol which was created with the assistance of an international steering committee of nine highly experienced clinicians and researchers (Ivory et al., 2023, Ivory et al., 2025b). Salient points in regard to the design process were made in the discussion section of the protocol for this study (Chapter 3, section 3.4.2) and they will be made again here to emphasise how the contributions of the steering committee helped to reinforce the solidity of its methodological design (Ivory et al., 2023).

The reliability and validity of the findings of Delphi studies in general have been called into question for a number of reasons (Ivory et al., 2023). Firstly, the Delphi process has been taken up by multiple healthcare fields wherein it has been modified so extensively that a uniform process is rarely used by researchers and as of 2017, ‘clear recommendations on conduct of Delphi studies and a reporting standard for their publication in peer-reviewed journals to date were not available’ (Ivory et al., 2023, Keeney et al., 2001, Jandhyala, 2020, Hasson et al., 2000, Jünger et al., 2017). A narrative article from 2023 maintained that this was still the case

and there is an editorial from 2024 that discusses the need for rigour in consensus statements and guidelines, although this article is currently inaccessible through the University of Galway's library (Howell, 2024, Shang, 2023). In May of 2025 an article was published in which a Delphi study was carried out with the aim of developing guidelines for the design and execution of Delphi studies, but again this article is currently inaccessible and it is unlikely that these guidelines have yet been completed (Hohmann et al., 2025). Secondly, rather than having a standard guidance for selecting expert panellists and deciding on the size of a panel, common sense and practical logistics are often used instead and the impact that the composition of a panel has on study outcomes has not been investigated (Keeney et al., 2001, Williamson et al., 2017, Ivory et al., 2023). Thirdly, there is no apparent, agreed-upon set percent agreement figure although 80% is used in many studies (Ivory et al., 2023, Falzarano and Pinto Zipp, 2013).

To address these and other methodological issues, the steering committee was given a list of 11 items based on areas of Delphi Methodology reported in the Core Outcome Measures in Effectiveness Trials (COMET) handbook (Williamson et al., 2017, Ivory et al., 2023). The items were as follows: 1) number of panels; 2) panel size; 3) participant information; 4) number of rounds; 5) questionnaire structure; 6) scoring methods; 7) between-round feedback; 8) criteria for outcome retention; 9) attrition; 10) definition of consensus; 11) means for assessing consensus, and the committee members were asked to bring their considerable experience and knowledge to bear on each item and refine it into a rigorous, practical and feasible methodological step (Ivory et al., 2023). So for example, the decision to use two rounds in this study was a result of discussing the trade-off between a classical methodological ideal of four rounds (Keeney et al., 2001), and the limited available time to execute the study plus the risk of panellists withdrawing from the process early because it was too lengthy and time consuming. For the panel composition, the committee had to decide between recruiting biomedical researchers only, a clinician group only, a mixed group of biomedical researchers and clinicians or two separate clinical and biomedical panels (Ivory et al., 2023). A series of arguments and questions for each scenario was presented, for example that a panel of biomedical researchers would be more familiar with the relevant (biofilm) literature, how immersion in relevant research might influence their perspective and how applicable laboratory-based research knowledge is to the clinical experience of wounds (Ivory et al., 2023). For a clinical panel it was argued that the outcome of this research was intended for clinicians and they might therefore have a greater vested interest in participating, but that they might not be so up-to-date on the relevant literature (Ivory et al., 2023).

The committee initially discussed clinical vs. biomedical research perspectives and attempted to decide between having a single clinical panel or two separate clinical and biomedical research panels (Ivory et al., 2023). It was argued for example that it can be difficult to apply academic or laboratory knowledge to a clinical context without relevant clinical experience and that the fruits of this research would serve clinical personnel exclusively (Ivory et al., 2023). Conversely, it was thought that although two separate panels might result in divergence of opinion, all opinions matter (Ivory et al., 2023). Ultimately, after dealing with arguments such as the above, the committee agreed to a panel comprised primarily of clinicians but also open to those clinicians who were experienced in research in the field. It was thought by all concerned that such a panel would best meet our research needs (Ivory et al., 2023).

Now that the importance of the steering committee's efforts in designing a rigorous methodology for this study have been discussed, the significance of the study itself in terms of clinical signs and symptoms of biofilm in chronic wounds can next be addressed.

This global study is thought to be the first of its kind in several ways. Compared to other works that have investigated this topic in a similar manner, that is other consensus or survey work, its panel was the largest with 103 individuals consenting to participate versus 81 and 14 in the other works (Metcalf and Bowler, 2016, Metcalf and Bowler, 2019, Haesler et al., 2019). It also had the most geographically and professionally diverse membership with 22 countries represented versus four as reported in one of the studies (Metcalf and Bowler, 2016, Metcalf and Bowler, 2019), and a panel consisting of podiatrists, nurses, doctors and other specialists vs. nurse practitioners in one study (Metcalf and Bowler, 2019, Metcalf and Bowler, 2016), and a panel chosen from a group that specialises in wound infection in the other (Haesler et al., 2019). The panel membership for this study was also highly competent, with the majority having more than 10 years' experience in a wound care environment. In addition, this study is more exclusive from a subject matter point of view than other works (Metcalf and Bowler, 2016, Metcalf and Bowler, 2019, Haesler et al., 2019), that is to say it concerned itself solely with clinical signs and symptoms of biofilm in chronic wounds, meaning that likely busy and time conscious panel members did not have to divide their attention across multiple topics. Other related studies presented topics such as defining an acute wound, defining biofilm, clinical indicators of a chronic wound, impact of biofilm on wound healing and experience with biofilm in addition to clinical signs and symptoms of biofilm to their participants (Haesler et al., 2019, Metcalf and Bowler, 2019, Metcalf and Bowler, 2016).

Interestingly, this study was subject to some controversy around the time of its circulation, which it was able to navigate successfully. Briefly, an invitation to participate email containing a PIL and link to the online questionnaire was sent to gatekeepers of four wound care organisations. Upon receiving the email, a committee member of one these organisations voiced protest and declared that the study was wrongly designed and that its findings would not be useful. The member also stated that the study was built on the wrong premises (this member is not of the opinion that biofilm delays wound healing). The concerns were addressed by this author via email but were never fully resolved – it is thought that the committee member was under the impression that the study operated in isolation, not as a part of a larger project. However, the wound care organisation committees continued to offer full support to the study and allowed it to proceed to completion. The decision to continue made by these organisations was doubtless a result of the methodological integrity of this study.

As with the scoping review, meaningful points and observations were presented in the discussion section of the published manuscript for this electronic Delphi study that need to be presented here once more because again, they lend strong credence to this PhD project.

It is difficult to say why the panel for this study voted on the included sign and symptom items the way they did. Members had the option to justify voting decisions in round one, but to discern patterns in this data would require a qualitative analysis which was not laid out in the study protocol (Ivory et al., 2023). The visual indicators item received the highest percentage of votes to have it included in the final, refined list despite the associated controversy which has been described previously in subsection 2.10.6. Biofilms in chronic wounds are a complex phenomenon, but humans are, according to the simplicity principle or Occam's razor, biased toward simpler, more straightforward explanations of phenomena and if this is true, then perhaps the idea of reducing the wound biofilms down to a more easily explainable, detectable and manageable phenomenon such as an observable layer on a wound surface that is easily removed and managed with regular debridement and cleansing becomes more understandable (Feldman, 2016). Six items that were voted for inclusion were relatable to infection, wound chronicity and failure to heal (failure to respond as expected to antimicrobial therapies; infection lasting more than 30 days/persistent cycles of recurrence [waxing and waning]; wound failing to progress to healing despite optimal management; wound duration of more than six weeks; soft tissue deterioration despite therapy and signs of local infection). Panellists may have voted as they did on these items because it is consistently reported in the literature that biofilm is associated with delayed healing. Looking at the 15 items upon which no

consensus was achieved, six or 40% (increased exudate; malodour; swelling/wound bed with pale oedema; temperature; wound pain/pain at site and low-level/increased erythema) constituted individual signs of local infection versus three or 27% (poor quality granulation tissue for example, discoloured, friable, fragile or hypergranulation; tunnelling/undermining and prolonged/persistent inflammation) of the 11 items that were voted for inclusion (Swanson et al., 2022). A study from 2009 that looked at validation of clinical signs of bacterial burden in chronic wounds concluded that ‘no one clinical sign may be sufficient to help clinicians decide whether a topical or systemic antibiotic should be prescribed’ (Woo and Sibbald, 2009). In addition it found that sensitivity was increased by combining any three signs (Woo and Sibbald, 2009). It may be that clinicians are more accustomed to conceptualizing signs of infection as a complete entity rather than thinking in terms of individual signs and symptoms. Other sign and symptom items on which no consensus was reached may have been seen as overly vague, or too focused on the whole patient rather than just the wound for example, other patient history indicators (change in the patient’s overall wellbeing or quality of life); extreme tolerance to host immune defence and history of systemic antibiotics in the last 12 months/prior or current use of antimicrobials, and thus not innately associated with a local wound context. One item was surprisingly not eliminated; sepsis is defined as a ‘life-threatening organ dysfunction caused by a dysregulated host response to infection’ and is an extreme, acute complication of infection that overwhelms the body’s systems that can be fatal (Singer et al., 2016).

One could continue to speculate as to why panellists voted the way they did. The point here though is that while we can’t ever be sure why, we can be sure from the findings of the scoping review that the reasons participants voted the way they did was likely not based on strong validation evidence (Ivory et al., 2025a).

Discussing this study in the context of other relevant literature, there are two other survey/consensus studies that have investigated signs and symptoms of biofilm in chronic wounds. Metcalf and Bowler carried out a market research study of 81 nurse practitioners from Germany, The USA, The UK and Italy (Metcalf and Bowler, 2016, Metcalf and Bowler, 2019). One of the questions in this survey asked how participants knew if a wound contained biofilm. There were 43 responses to that question and of these, 20 (46.5%) said that it is sometimes visible while 8 (18.6%) said that ‘it was not possible to see biofilm sometimes’ or ‘suggested indirect indicators of wound biofilm that is otherwise microscopic’ (Metcalf and Bowler, 2016, Metcalf and Bowler, 2019). Fifty-nine (90%) of the respondents in this Delphi study voted

visual indicators to be ‘very likely to be indicative of biofilm in chronic wounds’ whereas 3 (4.6%) who assigned a vote of ‘not likely to be indicative of biofilm in chronic wounds. A second question in Metcalf and Bowler’s survey asked about the impact of biofilm on wound healing of which 13 of 37 respondents (35%) referred to antimicrobial tolerance (Metcalf and Bowler, 2016, Metcalf and Bowler, 2019). In this Delphi study 50 (86%) of panellists who responded to the item relating to failure of the wound to respond as expected to antimicrobial therapies voted it as ‘very likely to be indicative of biofilm in chronic wounds’ and one (1.7%) voted it as ‘not likely to be indicative of biofilm in chronic wounds.’ On a frequency chart of indirect signs in the Metcalf and Bowler study, ‘poor responses to standard care, including antimicrobial dressings or antibiotics ranked highest (Metcalf and Bowler, 2016, Metcalf and Bowler, 2019). Other items on the chart included ‘delayed healing’, ‘exudate’, ‘odour’, and ‘inflammation’ (Metcalf and Bowler, 2016, Metcalf and Bowler, 2019). Similarly, the item pertaining to failure of a wound to heal despite optimal management strategies was voted ‘very likely to be indicative of biofilm in chronic wounds’ in this Delphi study.

A Delphi study by Haesler et al. queried clinical signs as indicators of biofilm in chronic wounds (Haesler et al., 2019). In their study, panel members voted contrary to those of this PhD’s study on a visual indicator item phrased as a ‘surface substance on the wound that can be visualised is an indicator of potential biofilm in a wound’ over two rounds. Specifically, that item received median (30-70% inter percentile range [IPR]) scores of 3.5 (1.7–5.6) and 2.0 (1.4-2.0) for rounds one and two respectively on a 9-point Likert scale similar to that used in this PhD’s study (Haesler et al., 2019). The Median (interquartile range) score for the corresponding item in this PhD’s Delphi study was 9.0 (1). The panel membership for the two Delphi studies was different in terms of size and probably more significantly, composition. Panellists in the Haesler study were recruited from an international group specialising in wound infection whereas this PhD’s study brought in nurses, doctors and podiatrists based primarily on their experience in a wound care environment (Haesler et al., 2019). It is possible that a small group of clinical and research personnel chosen from a group that specialises in wound infection management could have greater familiarity with current trends in that area and thus be more critical. Conversely, five of the remaining ten items in this PhD’s study (failure to respond as expected to antimicrobial therapies; prolonged/persistent inflammation; signs of local infection; poor quality granulation tissue for example, discoloured, friable, fragile or hypergranulation and wound failing to progress to healing despite optimal management)

corresponded with items that received a vote of ‘agreement’ in the Haesler et al. study (Haesler et al., 2019).

When viewed in the context of similar opinion work on the topic of which biofilm signs and symptoms point to biofilm in chronic wounds then, there does not appear to be complete coherence between groups of wound care personnel. This important observation lends even more support to the importance of a diligent effort to explore the true statistical abilities of reported clinical signs and symptoms to predict the presence of biofilm in chronic wounds.

In summary, the Delphi consensus phase of this PhD project has achieved its objective. It took a list of 26 clinical sign and symptom items generated by the scoping review and refined it down through experienced, international clinical opinion to 11 items. In a clinical setting, a predictive sign and symptom tool comprising 26 items would be too cumbersome and time consuming to be practical and manageable. A list of 11 items would be much more realistic and user friendly in this context. It is the largest, most geographically diverse and most comprehensive and topically specific survey-type study yet in terms of biofilm signs and symptoms in chronic wounds, and with its scale and the diversity and experience of its panel coupled with the rigour of its methodology, its findings can be taken as trustworthy with excellent external validity. Furthermore, the ESGB board concluded in a 2023 article that ‘there is a real need for survey articles discussing new methods of diagnosis and treatment of biofilm infections in order and this data could possibly be of service (Høiby et al., 2023).

The study could be critiqued on the basis of attrition with completion rates for rounds one and two of 54% and 92% respectively. However, sample sizes across Delphi studies have been reported to range from 12 to greater than 300 and the number of completers for each round in this study fall within that range (Ivory et al., 2023, Williamson et al., 2017). In addition, the guidance that directed the methodology for this study reported a response rate of 80% to be adequate in most situations but that there is no standard acceptable rate (Williamson et al., 2017). Twenty-five panel members initiated round two and 23 completed which account for 83% and 77% of the sample size of at least 30 which was deemed by the steering group to be optimal for this work.

It was thought that limiting the study to two rounds would mitigate attrition to some degree. Were this study to be repeated this author would recommend using the same methodological protocol but perhaps adding an incentive such as entry to a prize draw for panel members who complete both rounds to the end with the aim of maintaining participation right to the end of the questionnaire. In addition, of 18 wound care organisations around the world that were

approached, only four responded and agreed to facilitate this study. Time constraints for this work meant that these organisations could not be pursued indefinitely. Perhaps if more resources were to be devoted to this aspect of the study in a repeat running, a more extensive initial panel membership could be gained.

At this point the scoping review and electronic Delphi phases of this PhD project have been discussed in depth. Their methodologies have been presented in terms of their rigour and the efforts put into their design and conduct, and these methodologies have also been critiqued. Their findings have also been presented in the context of related literature and significantly, important observations arose from this presentation of findings, which add more justification for the work proposed and initiated in this project.

The project phases subsequent to the scoping review and electronic Delphi studies will now be discussed.

4.3.3 The clinical study protocol

The electronic Delphi phase of this PhD project refined the 26 sign and symptom item list resulting from the scoping review phase into a final list of 11 clinical sign and symptom items, and these have been incorporated into a protocol for a clinical study. This study protocol was created with the aim of statistically determining the abilities of these final 11 items to predict the presence of biofilm in chronic wounds by calculating sensitivity, specificity, discriminatory power and positive predictive value for each one. Very simply, the wounds of consenting patients will be assessed for dichotomous presence or absence of each item. Each wound assessment will be matched with a gold standard biopsy taken from the same wound and assessed for dichotomous presence or absence of biofilm by means of SEM and a fluorescent dye staining technique in conjunction with CLSM.

The protocol was carefully and thoughtfully designed with the focus primarily on ensuring that the rights, safety and wellbeing of study participants be protected as per ICH-GCP (International Council for Harmonisation, 2025), followed closely by efforts to guarantee that the study would have optimal methodological integrity.

Ethical approval for the protocol was sought and obtained from GCREC in June of 2024 and an amendment to the protocol received board approval in December of 2024. Copies of the approval letters from that board are included in Appendix 3. Originally it was planned to carry

out the study at the Diabetes Centre, UHG, but following a suspension of all research activities at that location, the study had to be relocated to an alternative site. This explains why initial approvals come from GCREC. With respect to the initial submission, the board raised no concerns in regard to the recruitment and consent processes, the wound assessment process, the biopsy procedure, the distress protocol, the patient follow up process or the clinician inter-rater reliability process. It did however request a single change to the 'Ethical concerns and data protection' section, requesting that cloud storage be used to transfer data from the investigative site to the University of Galway rather than a USB device. Once this issue was rectified, the board granted full approval for the study to proceed.

The amendment to the protocol which received immediate board approval was made to the biopsy procedure. The original procedure was guided by a clinical management article by Alavi et al. (Alavi et al., 2010), but after consultation with personnel from the Department of Dermatology, UHG some minor alterations were made to enhance patient safety and ensure that the procedure was carried out in accordance with local practice. These alterations included changing the biopsy size from 3mm diameter x up to 10mm depth to 4mm diameter x up to 5mm depth and changing the post biopsy repair procedure from using sutures to using local pressure with gauze followed by an extra pressure layer over usual wound dressing for 48 hours. No study can proceed without full approval granted by research ethics committee. This particular protocol involves a somewhat invasive procedure carried out on lower limb ulcers in a population that typically has compromised healing abilities in the lower extremities. Maximal effort was therefore made to address all potential ethical and safety concerns that could affect this population prior to submission of the protocol to the board. The fact that GCREC had no queries about the protocol other than the single item related to data storage and transfer is testament to that effort, made during the protocol's creation stage.

Methodologically speaking, the study design is not original; the CPR approach for items used in screening tools 'require(s) evidence of criterion-referenced validity in which the score of the clinical prediction rule has a strong relationship to a "gold standard" that identifies the presence or absence of the condition. This gold standard is often derived from information obtained from imaging, biopsy or surgery' (Beattie and Nelson, 2006). This, however, is the first time to this author's knowledge that such a protocol has been designed with the specific purpose of explicitly applying the CPR approach to clinical signs and symptoms thought to be indicative of biofilm in chronic wounds. The study visit for a single patient was first imagined and visualised based on this author's previous experience in clinical study design, on the methods

used in published reports of validation studies in other fields (Gardner et al., 2001, Gardner et al., 2007, Blodgett, 2013), and with the derivation stage of the CPR approach incorporated as a framework. The visualisation began at the point of first contact with a patient, that is introducing the study, through the consent and study procedures, to applying the patient's dressing at the end of the visit and follow up with the clinic and primary care providers. The individual steps were then written up with each step receiving careful critical appraisal for methodological flaws and potential for bias before finalising. Advice from specialists in microbiology, microscopy and dermatology were sought for the imaging work and the biopsy procedure, and a blinding scheme was incorporated to minimise observer/detection bias (Mansournia et al., 2017, Núñez and Matthews, 2025).

The sample size is small ($n = 12$) for good reason and was justified in the protocol (Chapter 3, section 3.5.2, 'sample size'). To the knowledge of this author, a study of comparable design and methodology to this has not been previously executed in the context of biofilm signs and symptoms in chronic wounds. It would therefore be prudent to carry out this work on a smaller scale than would normally be required for CPR development work to assess its feasibility and elucidate its weaknesses and strengths before committing to a full-scale more resource intensive iteration. Furthermore, much of this initial feasibility work will be carried out on a goodwill basis and no budget is required. However, the average study-related costs considering study materials such as dressings, anaesthetic, syringes/needles, punch biopsies, sample containers etc. will be estimated for a single patient in this work, and this data will be used to develop budgets for larger study iterations.

The SEM process in this process is standard, recommended for detecting biofilm in wound biopsy tissue and it has been used in other studies to assess both wound biopsy tissue and debridement samples for biofilm (Oates et al., 2014, Høiby et al., 2015, Malone et al., 2017b, Johani et al., 2017). The fluorescence imaging section is novel however and while shown to be effective *in vitro*, it would ideally be calibrated in *ex vivo* samples for example biopsies taken from pig skin inoculated with biofilm producing bacterial isolates prior to using it in human wound biopsy tissue. This technique will be reviewed in the next section of this discussion chapter.

In the context of other research work, three other studies have attempted to determine the abilities of various clinical signs and symptoms to indicate presence of biofilm in chronic wounds (Hurlow et al., 2016, Johani et al., 2017, Mayer et al., 2024). These have been discussed in more detail in the scoping review phase section of this discussion and some concerns in terms

of their methodologies were highlighted. Briefly, validation work on clinical signs and symptoms was preliminary or constituted secondary outcomes in those studies, sample sizes were small and the gold standard wound biopsy recommended by ESCMID guidelines was only used in one study (Høiby et al., 2015, Johani et al., 2017). Furthermore, diagnostic statistical tests were inconsistent across studies, indeed non-existent in one (Hurlow et al., 2016), as were the clinical signs and symptoms under assessment – one study assessed a single visual sign (Hurlow et al., 2016), another assessed a series of six (Johani et al., 2017), and the third apparently assessed signs and symptoms as a collective entity (Mayer et al., 2024). When viewed alongside these other studies, this protocol appears to overcome many of the methodological concerns highlighted above. Assessing the abilities of clinical signs and symptoms to predict biofilm in chronic wounds is its primary outcome, a gold standard biopsy will be taken from consenting participants, appropriate diagnostic statistical testing has been selected, and a comprehensive and refined list of 11 clinical signs and symptoms will be assessed.

In summary, this is an ethically sound study with a robust methodology. It has strong foundations in well-conducted scoping review and electronic Delphi studies. These studies supplied it with a comprehensive list of clinical signs and symptoms reported in the literature to be indicative of biofilm in chronic wounds that were refined through appropriately experienced opinion to a clinically manageable list of 11 items. Its design was diligently conceptualised, based on previous experience and validation work from other fields, and grounded in the CPR approach. It is currently set up as a feasibility study which will pave the way for subsequent iterations which will have the potential to recruit much larger sample sizes more appropriate to the CPR approach. This protocol will be instrumental in helping this PhD project achieve its aims.

4.3.4 Investigative site access

The original intent for this PhD project was to carry out a preliminary clinical study in which the list of 11 clinical signs and symptoms thought to be indicative of biofilm in chronic wounds that resulted from the electronic Delphi study would be statistically assessed for their true predictive abilities against a gold standard biopsy taken from lower limb ulcers in a sample of consenting patients with type 2 diabetes. This study was intended to be run on a small scale so that unanticipated complications and barriers to its successful completion could be identified and resolved, thus paving the way for larger, more successful iterations in the future.

A protocol was created for this study that received favourable ethical approval from GCREC and much of the groundwork in preparation for hosting the study at the Diabetes Centre investigative site at UHG had been laid. However, for logistical reasons all research projects at that site were suspended, and an alternative location thus had to be found. Colleagues at the Diabetes Day Centre, St. James's Hospital Dublin offered to facilitate the study and engagement with ethical, regulatory and data protection systems were subsequently initiated to permit fully approved access to that site.

This author has prior experience with clinical trials in wound care which by now though is somewhat outdated, and in the light of this experience it was anticipated that the route would be relatively simple and that the study would proceed to completion in a timely manner. However, as the route began to unfold, it became apparent that this would not be the case. The regulatory, ethical and data protection systems for an inter-institutional study such as this study require that a researcher engages with six different offices. St. James's hospital has a Research and Innovation office and a Joint Research Ethics Committee with Tallaght University Hospital. The University of Galway has a Trials Accelerator Group, a Clinical Research and Development Office, an Insurance Services Office, and a Data Protection Office. The functions of each of these offices and the interplay between them has been described in full in Chapter 3, section 3.6, subsections 3.6.4 and 3.6.5 of this thesis. Each of the offices has its own web page where they advertise information about themselves such as their purpose, services and training. On the University of Galway website, this information is typically divided into category panes presented on the offices' main pages and accessed with a click of the mouse. The offices are versatile in terms of their services and for the most part they have a lot of information to present; in some cases, clicking on a category pane guides the user to another layer of subcategories with even more information. When viewed collectively, the volume of information provided can seem overwhelming to the extent that researchers, especially time-deprived and pressurised novice or student researchers can get lost in the maze and much valuable information can be overlooked. For example, this author did not become aware of the Trials Accelerator group, an advisory service that supports researchers in the initial phases of clinical trial development until he was directed to it by personnel from another regulatory office despite the fact that it has its own web page. By the time contact was made with this office much of the work in terms of developing this study, work with which this office serves to assist, had already been completed. In retrospect, a journey through these systems by this uninitiated author could be equated to attempting to unravel a tangle of ropes whilst wearing a blindfold. There is no clear starting

point but feeling a random loose end and pulling on it will unravel the tangle somewhat and reveal another loose end, which when pulled on, will unravel the tangle some more and so forth. The equivalent of pulling on loose ends in this context would be discovering an office in an institution, often randomly and in the absence of a clear starting point, wherein its personnel might instruct on required documentation or help open the door to another office and so forth. Thus, the systems started to unravel and little by little their machinations became clearer. For this study those first loose ends were the SJH/TUH JREC and the University of Galway DPO. This journey began in the third quarter of 2024 and as of September 2025 it is still ongoing. Although disappointing in terms of timelines and regulatory pathways, the process did result in an excellent learning experience that yielded essential insights into clinical trial administration that all researchers should have. Furthermore, it resulted in an unanticipated but valuable research output. A novel, easy to follow, helpful and timesaving guidance document was created along with a route map/schematic documenting a more efficient and streamlined passage through these regulatory, ethical and data protection systems. This guidance will be of great assistance to novice researchers such as PhD students who are engaging in clinical research for the first time and are unfamiliar with these systems. Indeed, it has been reviewed by the Trials Accelerator group of the University of Galway's Institute for Clinical Trials. The group suggested no revisions and accepted the first draft as it was written with a plan to incorporate it into their future practices.

On viewing the journey outlined above in the context of modern clinical research, a significant issue becomes obvious. Much of what exists today by way of the regulatory, ethical and data protection systems governing this research have their origins in dark and abusive research events that occurred relatively recently which have been described in chapter 3, section 3.6, subsection 3.6.1. These systems are thus a vital part of clinical research involving human participants, ensuring that their rights safety and wellbeing are guaranteed and protected. Furthermore, they ensure that researchers remain mindful of the fact that the participant must always take precedence over the research at hand and any benefits that it may provide to society. Previous experience of working with patients in a wound care clinic that was also an investigative site for clinical trials means that this author intuitively puts the participant first in any research endeavour, attempting to do so to a fault. The protocol for this study was written primarily with participants' rights, safety and wellbeing in mind and full review by a Research Ethics Committee prior to initiating this inter-institutional journey to investigative site access deemed it to be ethically sound. In addition, the data protection processes were carefully

written, and every effort was made to minimise the risk of a data breach, and it is obvious that access to participant data for this study by unauthorised individuals would be almost impossible. The process was not faultless initially, but the REC highlighted a potential for a data breach, and this was resolved prior to final board approval.

This protocol is not unusual in this respect, nor is this author. The vast majority of researchers are fully aware of the ethical implications of their research and are highly conscientious and observant of applicable guidelines and regulations. Nevertheless, and this is the significant issue, gaining access to an investigative site for this study has proven to be a lengthy and complex affair. It has been ongoing for over a year, and it means that this PhD project could not complete what it initially set out to do in a timely manner necessitating that its direction and aim change radically and suddenly.

It could be argued that the naivete of this author in regard to regulatory affairs for today's clinical trials was a factor in the time this journey has taken, but anecdotal accounts from other researchers suggests that their projects must navigate and experience similar complexities and delays. More anecdotal accounts indicate that researchers are moving away from primary research and gravitating toward secondary research projects (evidence synthesis) because this type of research is not subject to such complex oversight.

Furthermore, on examination of this authors previous experience with clinical research regulatory affairs dating back to the late 2000s, the processes seemed less complex then and the turnaround time for approvals shorter, yet the same strict ethical principles were rigorously upheld. This author does not remember any of the many clinical trials in wound care to which he was party needing more than a year to acquire all of the necessary approvals to allow them to proceed.

A question as to why routes to approval for research would appear be getting increasingly complex and lengthy thus raises itself. Is it because institutions feel the need to protect themselves ever more to the extent that institutional protection takes precedence over research participant protection? The stakes in research can be high, and penalties levied against institutions for the consequences of research errors and data breaches are significant, therefore efforts to protect against such adverse outcomes are understandable and indeed advisable. However, if evolving regulatory processes are turning primary research projects into overly time consuming and cumbersome ordeals, everyone loses out. Primary research is vital for innovation and development of new knowledge, without which medicine will fail to flourish

and advance. In addition, without primary research, secondary research will also falter and grind to a halt.

It is not the function of this PhD project to investigate regulatory dilemmas faced by researchers. However, this author's experience and the associated anecdotes are troubling. Future research could survey researchers to quantify the problems at hand and if they prove to be truly significant then the regulatory ethical and data protection systems may need exploring to see if they can be made more researcher friendly without compromising participant rights, safety and wellbeing or indeed without compromising institutional safety.

4.3.5 The fluorescence staining procedure

The process for using staining biofilm with fluorescent dyes and imaging them with CLSM has been presented in this thesis in the form of a manuscript prepared for submission to an international wound care journal. Relevant points made in the discussion section of the manuscript may be repeated because it emphasizes why this phase was undertaken and the importance of what it achieved.

This phase of the PhD project comprised a preliminary exploration of the possibility of using a series of fluorescent dyes in conjunction with CLSM to highlight physical attributes of biofilms with the aim of developing an imaging process to facilitate confirmation of biofilm in biopsy tissue taken from chronic diabetic foot ulcers.

European Society of Clinical Microbiology and Infectious Disease guidelines outline a series of physical attributes that can be used to characterise bacterial biofilms (Høiby et al., 2015). These are microbial cell aggregates, a self-produced, ECM and adherence to or association with natural surfaces within the host and physiological/biochemical gradients (Høiby et al., 2015). Previous studies concerned with identifying biofilm in biopsies of chronic wound tissue have typically chosen the first three and have mostly used a combination of SEM and PNA-FISH in conjunction with CLSM to highlight them (Malone et al., 2017b, Johani et al., 2017, Kirketerp-Møller et al., 2008). This PhD project had hoped to use the same procedures to outline these features in biopsy tissue taken from chronic wounds but the PNA FISH kits that other researchers had used in the past were now no longer commercially available and alternative options had to be considered (Lichtenberg et al., 2024, Fazli et al., 2009, Johani et al., 2017, Kirketerp-Møller et al., 2008). Colleagues in microbiology at the University of Galway's School of Biological and Chemical Sciences were consulted, and while the idea of reproducing the PNA FISH kits was discussed, given these colleagues' experience with fluorescence

microscopy it was decided to develop an approach using fluorescent dyes in conjunction with CLSM tailored for the purposes of this PhD project instead. Briefly, biofilms from Methicillin resistant *Staphylococcus aureus* (MRSA) and Methicillin susceptible *Staphylococcus aureus* (MSSA) isolates were grown *in vitro*, stained with a series of fluorescent dyes chosen for their specificity for DNA, biofilm polysaccharide-type and protein-type ECM and imaged with CLSM.

Combinations of DNA and protein or polysaccharide specific dyes used in this approach proved capable of highlighting both the microbial cell aggregate, and ECM features as described by the ESCMID guidelines and a series of clear, high quality images were obtained (Høiby et al., 2015). PNA FISH probes consist of synthetic nucleic acid sequences tagged with a fluorescent label (Malic et al., 2009). In the context of biofilms they can be tailored to be universal or species specific but either way they will only highlight bacterial cell aggregate features and a second imaging methodology such as SEM or a staining process is necessary to find ECM features (Malone et al., 2017b, Kirketerp-Møller et al., 2008, Johani et al., 2017, Thurnheer et al., 2004). It also proved to be straightforward and efficient. It was executed in five relatively simple steps compared to three for PNA FISH and it required 2.5 – 3 hours to complete for two samples which is comparable to the PNA FISH procedure (Kirketerp-Møller et al., 2008, Johani et al., 2017, Fazli et al., 2009). It is envisaged that the process could be easily scaled up to manage larger numbers of samples within a similar time frame.

The process achieved its aim successfully under *in vitro* conditions that consisted solely of bacterial cells. In the case of a tissue sample from a wound biopsy however, the specificity of PNA probes for bacterial cells hold the advantage over this fluorescent staining procedure as its stains for DNA material are non-specific, binding to adenine-thymine rich sequences which occur in both bacterial and host cell DNA (Kapuscinski, 1995). Nevertheless, the difference in size between host and bacterial cells is vast and because DNA is contained within the nucleus of eukaryotic host cells, void areas should be visible around those cell nuclei stained by this project's process thus permitting distinctions between host and bacterial cells to be made in CLSM images. Fluorescent stains for polysaccharide and protein used in this process are considered to be specific for biofilm ECM and so no confounding issues are anticipated in their regard.

The process had a small sample size and was uncontrolled. Yet it still demonstrated an ability to identify two physical attributes known to present in bacterial biofilms. The next stage in the development process will be to reproduce it under *ex vivo* conditions in which biopsies will be

taken from pig skin samples inoculated with MRSA and MSSA isolates. This stage will provide a more realistic context in which the concerns around the non-specific nature of the DNA fluorescent dyes can be addressed. It will also involve a larger number of samples, and it will be possible to have control samples consisting of biopsies taken from uninoculated areas of the pig skin samples.

In summary, the fluorescent staining procedure developed in this process phase arose out of a need to find a timely replacement to the PNA FISH process, the prefabricated kits for which could not be obtained commercially. The procedure has proven itself provisionally successful - capable of highlighting morphological features of microbial biofilms in an *in vitro* context. The next steps should involve *ex vivo* work which will present a more realistic scenario with a mix of host and bacterial cells in which concerns around non-specificity of fluorescent dyes, small sample size and control groups can be addressed. It is cautiously anticipated that this process will show itself to be similarly effective under these conditions.

4.4 Chapter summary

In this chapter gaps in the knowledge that gave rise to the rationale for this PhD project and its aims and objectives were restated. The findings of each of the methodological phases of this work were subsequently summarised together, demonstrating the interdependence of each phase upon the others. Phase findings were then discussed individually in the context of lessons learned, in the context of the literature and in the context of today's clinical research processes where appropriate. Observations made during exploration of the findings of the phases were also presented, especially those that lend greater credence to the aim of this PhD project.

It is hoped that the reader now has a more comprehensive understanding of what this PhD project set out to do, what was done, how it was achieved and what it has contributed. If this has been achieved, the discussion chapter can be considered complete, and the reader will next be taken to the conclusion section of this PhD project.

4.5 Thesis Conclusion

There are concerns regarding strategies and interventions used to treat biofilm in chronic wounds in terms of their efficacy and good antimicrobial stewardship that arise from the mostly *in vitro* evidence supporting their use and the lack of routine, clinically available diagnostic tests to confirm their presence. This PhD project aimed to address these concerns and direct current research towards an attempt to develop a predictive tool for biofilm based on statistically validated clinical signs and symptoms widely reported to indicate its presence in chronic wounds. Five distinct yet interlocking methodological phases worked in unison to successfully achieve this aim. Each meticulously designed and executed phase resulted in a reliable and valid research output that can make a valuable contribution to wound care research. However, when taken in unison, their contribution becomes much more significant. Clinical signs and symptoms are widely used to confirm biofilm presence in chronic wounds but work to statistically validate their confirmatory abilities is as yet inadequate and limits the possibility determining if they can truly be harnessed into a reliable and valid predictive tool.

This project has for the first time opened an avenue for research to channel itself into a large-scale clinical prediction rule project to see if such a tool can be developed. It has provided the wound care research community with tools including a rigorously refined list of 11 clinical signs and symptoms forged into an ethically sound and methodologically rigorous protocol for a clinical validation study, a guidance document and schematic map to help researchers navigate the complex and time consuming ethical, regulatory, and data protection systems that lead to approved investigative site access in a timely and efficient manner and a fluorescence-based imaging technique that has demonstrated an ability to highlight morphological features of biofilms under *in vitro* conditions, cautiously anticipated to be capable of demonstrating similar abilities under *ex vivo* conditions.

It is eagerly anticipated that these tools will be put to use sooner rather than later and that the wound care community will be left with a more definitive answer as to the true predictive abilities of clinical signs and symptoms reported in the literature to be indicative of biofilm in chronic wounds.

4.5.1 Recommendations for practice

- At this point in time the Data Protection Impact Assessment (DPIA) required by the University of Galway's Data Protection Office (DPO) is still under review and must be completed. This assessment is a key component in terms of the regulatory, ethical and data protection systems that govern approval for clinical studies and as long as it holds this status, the study cannot progress through these systems.
- The colleague at the Diabetes Day Centre, St. James' Hospital who initially agreed to facilitate this study and indeed who would have personally carried out many of its methodological procedures has left the centre. A new contact may need to be made there if the study is to proceed.
- The fluorescence imaging procedure developed during this study has shown itself capable of identifying biofilm morphological features *in vitro*. It is cautiously anticipated that it will be capable of doing the same in wound tissue biopsy samples. Before committing to using the procedure in human *ex vivo* samples, it is recommended that the procedure be first investigated in animal *ex vivo* samples such as biopsies taken from pig skin tissue inoculated with biofilm forming bacterial strains such as Methicillin Resistant and Methicillin Susceptible *Staphylococcus aureus*. This will allow control groups to be established and a larger sample size to be investigated.
- At this point it is unknown whether the 11 clinical signs and symptoms derived in this research have the ability to predict presence of biofilm in chronic wounds from a statistical perspective. If the initial small-scale study proves methodologically feasible and a larger scale validation study is initiated, that study should specify cutoff values in terms of sensitivity, specificity, positive predictive value etc., below which a sign or symptom can be considered unfit for purpose. This would help to minimise research waste by providing definitive endpoints to determine whether the research should proceed or be terminated.

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Appendices

Appendix 1: Phase 1

- Search strategies for:
 - The Medline database p. 262
 - The CINAHL database p. 265
 - The Embase database p. 268
 - The Cochrane Central database p. 271
 - The Bielefeld Academic Search Engine (BASE) p. 274

- A completed Peer Review of Electronic Search Strategies (PRESS) assessment form p. 276

- A table of included studies p. 287

- A table of excluded studies p. 308

Medline

Run 13 Jan 22

1733 records

Re-run 06 July 23

466 records

1. "wounds and injuries"/
2. Wound healing/
3. Skin ulcer/
4. Leg ulcer/
5. Varicose ulcer/
6. Foot ulcer/
7. Diabetic foot/
8. Diabetes Mellitus, Type2/co [Complications]
9. Diabetic neuropathies/
10. Peripheral nervous system diseases/
11. Peripheral arterial disease/
12. Pressure ulcer/
13. Wound infection/
14. Debridement/
15. Re-epithelialization/
16. (chronic adj3 ulcer*).tw.
17. (skin adj3 ulcer*).tw.
18. (vascular adj3 ulcer*).tw.
19. (varicose adj3 ulcer*).tw.
20. (venous adj3 ulcer*).tw.
21. (stasis adj3 ulcer*).tw.
22. (leg adj3 ulcer*).tw.
23. (foot adj3 ulcer*).tw.
24. (diabetic adj3 ulcer*).tw.
25. (neuropathic adj3 ulcer*).tw.
26. (isch?emic adj3 ulcer*).tw.
27. (neuro-isch?emic adj3 ulcer*).tw.
28. (neuroisch?emic adj3 ulcer*).tw.
29. (pressure adj3 ulcer*).tw.
30. (decubitus adj3 ulcer*).tw.
31. (arterial adj3 ulcer*).tw.
32. (mixed adj3 ulcer*).tw.

33. (care adj3 ulcer*).tw.
34. (heal* adj3 ulcer*).tw.
35. (nonheal* adj3 ulcer*).tw.
36. (non-heal* adj3 ulcer*).tw.
37. (“non heal*” adj3 ulcer*).tw.
38. (re-epitheliali* adj3 ulcer*).tw.
39. (reepitheliali* adj3 ulcer*).tw.
40. (surface adj3 ulcer*).tw.
41. (“lower extremi*” adj3 ulcer*).tw.
42. (lower-extremi* adj3 ulcer*).tw.
43. (debrid* adj3 ulcer*).tw.
44. (manag* adj3 ulcer*).tw.
45. (bed adj3 ulcer*).tw.
46. (“hard to heal” adj3 ulcer*).tw.
47. (hard-to-heal adj3 ulcer*).tw.
48. (infect* adj3 ulcer*).tw.
49. (chronic adj3 wound*).tw.
50. (skin adj3 wound*).tw.
51. (vascular adj3 wound*).tw.
52. (varicose adj3 wound*).tw.
53. (venous adj3 wound*).tw.
54. (stasis adj3 wound*).tw.
55. (leg adj3 wound*).tw.
56. (foot adj3 wound*).tw.
57. (diabetic adj3 wound*).tw.
58. (neuropathic adj3 wound*).tw.
59. (isch?emic adj3 wound*).tw.
60. (neuro-isch?emic adj3 wound*).tw.
61. (neuroisch?emic adj3 wound*).tw.
62. (pressure adj3 wound*).tw.
63. (decubitus adj3 wound*).tw.
64. (arterial adj3 wound*).tw.
65. (mixed adj3 wound*).tw.
66. (care adj3 wound*).tw.
67. (heal* adj3 wound*).tw.
68. (nonheal* adj3 wound*).tw.
69. (non-heal* adj3 wound*).tw.
70. (“non heal*” adj3 wound*).tw.
71. (re-epitheliali* adj3 wound*).tw.
72. (reepitheliali* adj3 wound*).tw.
73. (surface adj3 wound*).tw.
74. (“lower extremi*” adj3 wound*).tw.
75. (lower-extremi* adj3 wound*).tw.
76. (debrid* adj3 wound*).tw.

77. (manag* adj3 wound*).tw.
78. (bed* adj3 wound*).tw.
79. ("hard to heal" adj3 wound*).tw.
80. (hard-to-heal adj3 wound*).tw.
81. (infect* adj3 wound*).tw.
82. (diabetic adj3 foot).tw.
83. (diabetic adj3 feet).tw.
84. (pressure adj3 sore*).tw.
85. bedsore*.tw.
86. "bed sore*".tw.
87. bed-sore*.tw.
88. or/1-87
89. ("mixed etiolog*" adj3 ulcer*).tw.
90. ("mixed aetiolog*" adj3 ulcer*).tw.
91. ("mixed etiolog*" adj3 wound*).tw.
92. ("mixed aetiolog*" adj3 wound*).tw.
93. or/89-92
94. 88 or 93
95. Exp Biofilms/
96. biofilm*.tw.
97. "EPS matrix".tw.
98. "EPS matrices".tw.
99. "extracellular polymeric substance*".tw.
100. or/95-99
101. 94 AND 100
102. Exp Animals/ NOT (Humans/ and exp Animals/)
103. 101 NOT 102

CINAHL

Run 19 Jan 22

715 records

Re-run 06 Jul 23

100 records

1. (MH "wounds and Injuries")
2. (MH "Wound Healing")
3. (MH "Skin Ulcer")
4. (MH "Leg Ulcer")
5. (MH "Venous Ulcer")
6. (MH "Foot Ulcer")
7. (MH "Diabetic Foot")
8. (MH "Diabetes Mellitus, Type2/CO")
9. (MH "Diabetic Neuropathies")
10. (MH "Peripheral Nervous System Diseases")
11. (MH "Peripheral Vascular Diseases")
12. (MH "Pressure Ulcer")
13. (MH "Wound Infection")
14. (MH "Debridement+")
15. (MH "Wound Assessment")
16. TI chronic N3 ulcer* OR AB chronic N3 ulcer*
17. TI skin N3 ulcer* OR AB chronic N3 ulcer*
18. TI vascular N3 ulcer* OR AB vascular N3 ulcer*
19. TI varicose N3 ulcer* OR AB varicose N3 ulcer*
20. TI venous N3 ulcer* OR AB venous N3 ulcer*
21. TI stasis N3 ulcer* OR AB stasis N3 ulcer*
22. TI leg N3 ulcer* OR AB leg N3 ulcer*
23. TI foot N3 ulcer* OR AB foot N3 ulcer*
24. TI diabetic N3 ulcer* OR AB diabetic N3 ulcer*
25. TI neuropathic N3 ulcer* OR AB neuropathic N3 ulcer*
26. TI isch?emic N3 Ulcer* OR AB isch?emic N3 ulcer*
27. TI neuro-isch?emic N3 Ulcer* OR AB neuro-isch?emic N3 ulcer*
28. TI neuroisch?emic N3 ulcer* OR AB neuroisch?emic N3 ulcer*
29. TI pressure N3 Ulcer* OR AB pressure N3 ulcer*
30. TI decubitus N3 Ulcer* OR AB decubitus N3 ulcer*
31. TI arterial N3 Ulcer* OR AB arterial N3 ulcer*
32. TI mixed N3 Ulcer* OR AB mixed N3 ulcer*
33. TI care N3 Ulcer* OR AB care N3 ulcer*
34. TI heal* N3 Ulcer* OR AB heal* N3 ulcer*
35. TI nonheal* N3 Ulcer* OR AB nonheal* N3 ulcer*
36. TI non-heal* N3 Ulcer* OR AB non-heal* N3 ulcer*
37. TI "non heal*" N3 Ulcer* OR AB "non heal*" N3 ulcer*
38. TI re-epitheliali* N3 Ulcer* OR AB re-epitheliali* N3 ulcer*
39. TI reepitheliali* N3 Ulcer* OR AB reepitheliali* N3 ulcer*

40. TI surface N3 Ulcer* OR AB surface N3 ulcer*
41. TI “lower extremit*” N3 Ulcer* OR AB “lower extremit*” N3 ulcer*
42. TI lower-extremit* N3 Ulcer* OR AB lower-extremit* N3 ulcer*
43. TI debrid* N3 Ulcer* OR AB debrid* N3 ulcer*
44. TI manag* N3 Ulcer* OR AB manag* N3 ulcer*
45. TI bed N3 Ulcer* OR AB bed N3 ulcer*
46. TI “hard to heal” N3 Ulcer* OR AB “hard to heal” N3 ulcer*
47. TI hard-to-heal N3 Ulcer* OR AB hard-to-heal N3 ulcer*
48. TI infect* N3 Ulcer* OR AB infect* N3 ulcer*
49. TI chronic N3 wound* OR AB chronic N3 wound*
50. TI skin N3 wound* OR AB chronic N3 wound*
51. TI vascular N3 wound* OR AB vascular N3 wound*
52. TI varicose N3 wound* OR AB varicose N3 wound*
53. TI venous N3 wound* OR AB venous N3 wound*
54. TI stasis N3 wound* OR AB stasis N3 wound*
55. TI leg N3 wound* OR AB leg N3 wound*
56. TI foot N3 wound* OR AB foot N3 ulcer*
57. TI diabetic N3 wound* OR AB diabetic N3 wound*
58. TI neuropathic N3 wound* OR AB neuropathic N3 wound*
59. TI isch?emic N3 wound* OR AB isch?emic N3 wound*
60. TI neuro-isch?emic N3 wound* OR AB neuro-isch?emic N3 wound*
61. TI neuroisch?emic N3 wound* OR AB neuroisch?emic N3 wound*
62. TI pressure N3 wound* OR AB pressure N3 wound*
63. TI decubitus N3 wound* OR AB decubitus N3 wound*
64. TI arterial N3 wound* OR AB arterial N3 wound*
65. TI mixed N3 wound* OR AB mixed N3 wound*
66. TI care N3 wound* OR AB care N3 wound*
67. TI heal* N3 wound* OR AB heal* N3 wound*
68. TI nonheal* N3 wound* OR AB nonheal* N3 wound*
69. TI non-heal* N3 wound* OR AB non-heal* N3 wound*
70. TI “non heal*” N3 wound* OR AB “non heal*” N3 wound*
71. TI re-epitheliali* N3 wound* OR AB re-epitheliali* N3 wound*
72. TI reepitheliali* N3 wound* OR AB reepitheliali* N3 wound*
73. TI surface N3 wound* OR AB surface N3 wound*
74. TI “lower extremit*” N3 wound* OR AB “lower extremit*” N3 wound*
75. TI lower-extremit* N3 wound* OR AB lower-extremit* N3 wound*
76. TI debrid* N3 wound* OR AB debrid* N3 wound*
77. TI manag* N3 wound* OR AB manag* N3 wound*
78. TI bed N3 wound* OR AB bed N3 wound*
79. TI “hard to heal” N3 wound* OR AB “hard to heal” N3 wound*
80. TI hard-to-heal N3 wound* OR AB hard-to-heal N3 wound*
81. TI infect* N3 wound* OR AB infect* N3 wound*
82. TI diabetic N3 foot OR AB diabetic N3 foot
83. TI diabetic N3 feet OR AB diabetic N3 feet
84. TI pressure N3 sore* OR AB pressure N3 sore*
85. TI bed N3 sore* OR AB bed N3 sore*
86. TI bedsore* OR AB bedsore*
87. TI bed-sore* OR AB bed-sore*
88. S1 OR S2 OR S3 OR S4 OR S5 OR S6 OR S7 OR S8 OR S9 OR S10 OR S11 OR S12
OR S13 OR S14 OR S15 OR S16 OR S17 OR S18 OR S19 OR S20 OR S21 OR S22

- OR S23 OR S24 OR S25 OR S26 OR S27 OR S28 OR S29 OR S30 OR S31 OR S32
OR S33 OR S34 OR S35 OR S36 OR S37 OR S38 OR S39 OR S40 OR S41 OR S42
OR S43 OR S44 OR S45 OR S46 OR S47 OR S48 OR S49 OR S50 OR S51 OR S52
OR S53 OR S54 OR S55 OR S56 OR S57 OR S58 OR S59 OR S60 OR S61 OR S62
OR S63 OR S64 OR S65 OR S66 OR S67 OR S68 OR S69 OR S70 OR S71 OR S72
OR S73 OR S74 OR S75 OR S76 OR S77 OR S78 OR S79 OR S80 OR S81 OR S82
OR S83 OR S84 OR S85 OR S86 OR S87
89. TI "Mixed etiolog*" N3 ulcer* OR AB "Mixed etiolog*" N3 ulcer*
90. TI "Mixed aetiolog*" N3 ulcer* OR AB "Mixed aetiolog*" N3 ulcer*
91. TI "Mixed etiolog*" N3 wound* OR AB "Mixed etiolog*" N3 wound*
92. TI "Mixed aetiolog*" N3 wound* OR AB "Mixed aetiolog*" N3 wound*
93. S89 OR S90 OR S91 OR S92
94. S88 OR S93
95. (MH "Biofilms")
96. TI biofilm* OR AB biofilm*
97. TI "EPS matri*" OR AB "EPS matri*"
98. TI "extracellular polymeric substance*" OR AB "extracellular polymeric substance*"
99. S95 OR S96 OR S97 OR S98
100. S94 AND S99

Limiters:

- Exclude MEDLINE records

Embase

Run 13 Jan 22

1268 records

Re-run 06 July 23

350 records

1. 'Wound healing'/de
2. 'wound healing impairment'/de
3. 'skin ulcer'/de
4. 'leg ulcer'/de
5. 'foot ulcer'/de
6. 'diabetic ulcer'/de
7. 'diabetic foot'/de
8. 'plantar ulcer'/de
9. 'diabetic neuropathy'/de
10. 'decubitus'/de
11. 'wound infection'/de
12. 'debridement'/exp
13. 'epithelialization'/de
14. (chronic NEAR/3 ulcer*):ab,ti
15. (skin NEAR/3 ulcer*):ab,ti
16. (vascular NEAR/3 ulcer*):ab,ti
17. (varicose NEAR/3 ulcer*):ab,ti
18. (venous NEAR/3 ulcer*):ab,ti
19. (stasis NEAR/3 ulcer*):ab,ti
20. (leg NEAR/3 ulcer*):ab,ti
21. (foot NEAR/3 ulcer*):ab,ti
22. (diabetic NEAR/3 ulcer*):ab,ti
23. (neuropathic NEAR/3 ulcer*):ab,ti
24. (isch*mic NEAR/3 ulcer*):ab,ti
25. (neuro-isch*mic NEAR/3 ulcer*):ab,ti
26. (neuroisch*mic NEAR/3 ulcer*):ab,ti
27. (pressure NEAR/3 ulcer*):ab,ti
28. (decubitus NEAR/3 ulcer*):ab,ti
29. (arterial NEAR/3 ulcer*):ab,ti
30. (mixed NEAR/3 ulcer*):ab,ti
31. (care NEAR/3 ulcer*):ab,ti
32. (heal* NEAR/3 ulcer*):ab,ti
33. (nonheal* NEAR/3 ulcer*):ab,ti
34. (non-heal* NEAR/3 ulcer*):ab,ti
35. ('non heal*' NEAR/3 ulcer*):ab,ti

36. (re-epitheliali* NEAR/3 ulcer*):ab,ti
37. (reepitheliali* NEAR/3 ulcer*):ab,ti
38. (surface NEAR/3 ulcer*):ab,ti
39. ('lower extremi*' NEAR/3 ulcer*):ab,ti
40. (lower-extremi* NEAR/3 ulcer*):ab,ti
41. (debrid* NEAR/3 ulcer*):ab,ti
42. (manag* NEAR/3 ulcer*):ab,ti
43. (bed NEAR/3 ulcer*):ab,ti
44. ('hard to heal' NEAR/3 ulcer*):ab,ti
45. (hard-to-heal NEAR/3 ulcer*):ab,ti
46. (infect* NEAR/3 ulcer*):ab,ti
47. (chronic NEAR/3 wound*):ab,ti
48. (skin NEAR/3 wound*):ab,ti
49. (vascular NEAR/3 wound*):ab,ti
50. (varicose NEAR/3 wound*):ab,ti
51. (venous NEAR/3 wound*):ab,ti
52. (stasis NEAR/3 wound*):ab,ti
53. (leg NEAR/3 wound*):ab,ti
54. (foot NEAR/3 wound*):ab,ti
55. (diabetic NEAR/3 wound*):ab,ti
56. (neuropathic NEAR/3 wound*):ab,ti
57. (isch*mic NEAR/3 wound*):ab,ti
58. (neuro-isch*mic NEAR/3 wound*):ab,ti
59. (neuroisch*mic NEAR/3 wound*):ab,ti
60. (pressure NEAR/3 wound*):ab,ti
61. (decubitus NEAR/3 wound*):ab,ti
62. (arterial NEAR/3 wound*):ab,ti
63. (mixed NEAR/3 wound*):ab,ti
64. (care NEAR/3 wound*):ab,ti
65. (heal* NEAR/3 wound*):ab,ti
66. (nonheal* NEAR/3 wound*):ab,ti
67. (non-heal* NEAR/3 wound*):ab,ti
68. ('non heal*' NEAR/3 wound*):ab,ti
69. (re-epitheliali* NEAR/3 wound*):ab,ti
70. (reepitheliali* NEAR/3 wound*):ab,ti
71. (surface NEAR/3 wound*):ab,ti
72. ('lower extremi*' NEAR/3 wound*):ab,ti
73. (lower-extremi* NEAR/3 wound*):ab,ti
74. (debrid* NEAR/3 wound*):ab,ti
75. (manag* NEAR/3 wound*):ab,ti
76. (bed NEAR/3 wound*):ab,ti
77. ('hard to heal' NEAR/3 wound*):ab,ti
78. (hard-to-heal NEAR/3 wound*):ab,ti
79. (infect* NEAR/3 wound*):ab,ti

80. (diabetic NEAR/3 foot):ab,ti
81. (diabetic NEAR/3 feet):ab,ti
82. (pressure NEAR/3 sore):ab,ti
83. (bed NEAR/3 sore):ab,ti
84. bedsore*:ab,ti
85. bed-sore*:ab,ti
86. #1 OR #2 OR #3 OR #4 OR #5 OR #6 OR #7 OR #8 OR #9 OR #10 OR #11 OR #12
OR #13 OR #14 OR #15 OR #16 OR #17 OR #18 OR #19 OR #20 OR #21 OR #22
OR #23 OR #24 OR #25 OR #26 OR #27 OR #28 OR #29 OR #30 OR #31 OR #32
OR #33 OR #34 OR #35 OR #36 OR #37 OR #38 OR #39 OR #40 OR #41 OR #42
OR #43 OR #44 OR #45 OR #46 OR #47 OR #48 OR #49 OR #50 OR #51 OR #52
OR #53 OR #54 OR #55 OR #56 OR #57 OR #58 OR #59 OR #60 OR #61 OR #62
OR #63 OR #64 OR #65 OR #66 OR #67 OR #68 OR #69 OR #70 OR #71 OR #72
OR #73 OR #74 OR #75 OR #76 OR #77 OR #78 OR #79 OR #80 OR #81 OR #82
OR #83 OR #84 OR #85
87. ('mixed etiolog*' NEAR/3 ulcer*):ab,ti
88. ('mixed aetiolog*' NEAR/3 ulcer*):ab,ti
89. ('mixed etiolog*' NEAR/3 wound*):ab,ti
90. ('mixed aetiolog*' NEAR/3 wound*):ab,ti
91. #87 OR #88 OR #89 OR #90
92. #86 OR #91
93. 'biofilm'/exp
94. biofilm*:ab,ti
95. 'EPS matrix':ab,ti
96. 'EPS matrices':ab,ti
97. 'extracellular polymeric substance*':ab,ti
98. #93 OR #94 OR #95 OR #96 OR #97
99. #92 AND #98

#99 AND [embase]/lim

Cochrane Central

Run 13 Jan 22

99 records

Re-run 06 Jul 23

128 records

- #1. MeSH descriptor: ["wounds and injuries"] this term only
- #2. MeSH descriptor: [Wound healing] this term only
- #3. MeSH descriptor: [Skin ulcer] this term only
- #4. MeSH descriptor: [Leg ulcer] this term only
- #5. MeSH descriptor: [Varicose ulcer] this term only
- #6. MeSH descriptor: [Foot ulcer] this term only
- #7. MeSH descriptor: [Diabetic foot] this term only
- #8. MeSH descriptor: [Diabetes Mellitus, Type2] this term only and with qualifier(s):
[complications – CO]
- #9. MeSH descriptor: [Diabetic neuropathies] this term only
- #10. MeSH descriptor: [Peripheral nervous system diseases] this term only
- #11. MeSH descriptor: [Peripheral arterial disease] this term only
- #12. MeSH descriptor: [Pressure ulcer] this term only
- #13. MeSH descriptor: [Wound infection] this term only
- #14. MeSH descriptor: [Debridement] this term only
- #15. (chronic NEAR/3 ulcer*):ti,ab,kw
- #16. (skin NEAR/3 ulcer*):ti,ab,kw
- #17. (vascular NEAR/3 ulcer*):ti,ab,kw
- #18. (varicose NEAR/3 ulcer*):ti,ab,kw
- #19. (venous NEAR/3 ulcer*):ti,ab,kw
- #20. (stasis NEAR/3 ulcer*):ti,ab,kw
- #21. (leg NEAR/3 ulcer*):ti,ab,kw
- #22. (foot NEAR/3 ulcer*):ti,ab,kw
- #23. (diabetic NEAR/3 ulcer*):ti,ab,kw
- #24. (neuropathic NEAR/3 ulcer*):ti,ab,kw
- #25. (isch?emic NEAR/3 ulcer*):ti,ab,kw
- #26. (neuro-isch?emic NEAR/3 ulcer*):ti,ab,kw
- #27. (neuroisch?emic NEAR/3 ulcer*):ti,ab,kw
- #28. (pressure NEAR/3 ulcer*):ti,ab,kw
- #29. (decubitus NEAR/3 ulcer*):ti,ab,kw
- #30. (arterial NEAR/3 ulcer*):ti,ab,kw
- #31. (mixed NEAR/3 ulcer*):ti,ab,kw
- #32. (care NEAR/3 ulcer*):ti,ab,kw
- #33. (heal* NEAR/3 ulcer*):ti,ab,kw
- #34. (nonheal* NEAR/3 ulcer*):ti,ab,kw
- #35. (non-heal* NEAR/3 ulcer*):ti,ab,kw
- #36. ("non heal*" NEAR/3 ulcer*):ti,ab,kw

- #37. (re-epitheliali* NEAR/3 ulcer*):ti,ab,kw
- #38. (reepitheliali* NEAR/3 ulcer*):ti,ab,kw
- #39. (surface NEAR/3 ulcer*):ti,ab,kw
- #40. (“lower extremi*” NEAR/3 ulcer*):ti,ab,kw
- #41. (lower-extremi* NEAR/3 ulcer*):ti,ab,kw
- #42. (debrid* NEAR/3 ulcer*):ti,ab,kw
- #43. (manag* NEAR/3 ulcer*):ti,ab,kw
- #44. (bed NEAR/3 ulcer*):ti,ab,kw
- #45. (“hard to heal” NEAR/3 ulcer*):ti,ab,kw
- #46. (hard-to-heal NEAR/3 ulcer*):ti,ab,kw
- #47. (infect* NEAR/3 ulcer*):ti,ab,kw
- #48. (chronic NEAR/3 wound*):ti,ab,kw
- #49. (skin NEAR/3 wound*):ti,ab,kw
- #50. (vascular NEAR/3 wound*):ti,ab,kw
- #51. (varicose NEAR/3 wound*):ti,ab,kw
- #52. (venous NEAR/3 wound*):ti,ab,kw
- #53. (stasis NEAR/3 wound*):ti,ab,kw
- #54. (leg NEAR/3 wound*):ti,ab,kw
- #55. (foot NEAR/3 wound*):ti,ab,kw
- #56. (diabetic NEAR/3 wound*):ti,ab,kw
- #57. (neuropathic NEAR/3 wound*):ti,ab,kw
- #58. (isch?emic NEAR/3 wound*):ti,ab,kw
- #59. (neuro-isch?emic NEAR/3 wound*):ti,ab,kw
- #60. (neuroisch?emic NEAR/3 wound*):ti,ab,kw
- #61. (pressure NEAR/3 wound*):ti,ab,kw
- #62. (decubitus NEAR/3 wound*):ti,ab,kw
- #63. (arterial NEAR/3 wound*):ti,ab,kw
- #64. (mixed NEAR/3 wound*):ti,ab,kw
- #65. (care NEAR/3 wound*):ti,ab,kw
- #66. (heal* NEAR/3 wound*):ti,ab,kw
- #67. (nonheal* NEAR/3 wound*):ti,ab,kw
- #68. (non-heal* NEAR/3 wound*):ti,ab,kw
- #69. (“non heal*” NEAR/3 wound*):ti,ab,kw
- #70. (re-epitheliali* NEAR/3 wound*):ti,ab,kw
- #71. (reepitheliali* NEAR/3 wound*):ti,ab,kw
- #72. (surface NEAR/3 wound*):ti,ab,kw
- #73. (“lower extremi*” NEAR/3 wound*):ti,ab,kw
- #74. (lower-extremi* NEAR/3 wound*):ti,ab,kw
- #75. (debrid* NEAR/3 wound*):ti,ab,kw
- #76. (manag* NEAR/3 wound*):ti,ab,kw
- #77. (bed NEAR/3 wound*):ti,ab,kw
- #78. (“hard to heal” NEAR/3 wound*):ti,ab,kw
- #79. (hard-to-heal NEAR/3 wound*):ti,ab,kw
- #80. (infect* NEAR/3 wound*):ti,ab,kw
- #81. (diabetic NEAR/3 foot):ti,ab,kw
- #82. (diabetic NEAR/3 feet):ti,ab,kw

- #83. (pressure NEAR/3 sore):ti,ab,kw
- #84. (bedsore*):ti,ab,kw
- #85. ("bed sore*"):ti,ab,kw
- #86. (bed-sore*):ti,ab,kw
- #87. #1 OR #2 OR #3 OR #4 OR #5 OR #6 OR #7 OR #8 OR #9 OR #10 OR #11
OR #12 OR #13 OR #14 OR #15 OR #16 OR #17 OR #18 OR #19 OR #20 OR #21
OR #22 OR #23 OR #24 OR #25 OR #26 OR #27 OR #28 OR #29 OR #30 OR #31
OR #32 OR #33 OR #34 OR #35 OR #36 OR #37 OR #38 OR #39 OR #40 OR #41
OR #42 OR #43 OR #44 OR #45 OR #46 OR #47 OR #48 OR #49 OR #50 OR #51
OR #52 OR #53 OR #54 OR #55 OR #56 OR #57 OR #58 OR #59 OR #60 OR #61
OR #62 OR #63 OR #64 OR #65 OR #66 OR #67 OR #68 OR #69 OR #70 OR #71
OR #72 OR #73 OR #74 OR #75 OR #76 OR #77 OR #78 OR #79 OR #80 OR #81
OR #82 OR #83 OR #84 OR #85 OR #86
- #88. ("mixed aetiology*" NEAR/3 ulcer*):ti,ab,kw
- #89. ("mixed aetiology*" NEAR/3 wound*):ti,ab,kw
- #90. #88 OR #89
- #91. #87 OR #90
- #92. MeSH descriptor: [Biofilms] explode all trees
- #93. (biofilm*):ti,ab,kw
- #94. #92 OR # 93
- #95. #91 AND #94

Bielefeld Academic Search Engine (BASE)

Advanced search function, search terms entered into “Entire document field”

Search 1: Chronic Wound biofilm

Run 18 Jan 22

1543 records reported, only 1000 physically presented in BASE for export, 1000 exported

Re-run 06 Jul 23

499 records

Search 2: Wound healing biofilm

Run 19 Jan 22

1640 records reported, only 1000 physically presented in BASE for export, 1000 exported

Re-Run 06 July 23

603 records

Search 3: Skin ulcer biofilm

Run 18 Jan 22

133 records reported, 133 records exported

Re-run 06 Jul 23

76 records

Search 4: Venous ulcer biofilm

Run 18 Jan 22

115 hits reported, 115 records exported

Re-run 06 Jul 23

35 records

Search 5: Diabetic foot biofilm

Run 18 Jan 22

358 hits reported, 358 hits exported

Re-run 06 Jul 23

Appendices

117 records

Search 6: Pressure ulcer biofilm

Run 18 Jan 22

135 hits reported, 135 hits exported

Re-run 06 Jul 23

17 records

Total: 2741

Re-run Total: 1,297

PRESS 2015 Guideline Assessment Form

SEARCH SUBMISSION: THIS SECTION TO BE FILLED IN BY THE SEARCHER

Searcher: John D. Ivory	Email: <u>J.IVORY2@nuigalway.ie</u>	
Date submitted: 28 July 21	Date requested by:	<i>[Max = 5 working days]</i>

Systematic review title:

A scoping review protocol to identify clinical signs, symptoms and biomarkers indicative of biofilm presence in chronic wounds
--

The search strategy Is....

√	My PRIMARY (core) database strategy – First time submitting a strategy for search question and database
	My PRIMARY (core) strategy– Follow-up Review NOT the first time submitting a strategy for search question and database. If this is a response to peer review, itemize the changes made to the review suggestions.
	SECONDARY search strategy– First time submitting a strategy for search question and database
	SECONDARY search strategy – NOT the first time submitting a strategy for search question and database. If this is a response to peer review, itemize the changes made to the review suggestions.

Database (i.e., MEDLINE, CINAHL,...)

[mandatory]

Medline

--

Interface (i.e., Ovid, EBSCO...)

[mandatory]

Ovid

Research question (Describe the purpose of the research)

[mandatory]

What clinical signs, symptoms and biomarkers are proposed withing the literature to determine the presence of biofilm in chronic wounds?
--

PICOS

format

(Outline the PICOS for your question, i.e., Patient, Intervention, Comparison, Outcome, and Study Design as applicable)

P	Adult patients with Venous leg ulcers (VLU), Diabetic foot ulcers (DFU), Pressure ulcers (PU), Mixed arterial/venous leg ulcers (MAVLU)
C	Clinical signs, symptoms or biomarkers currently used to confirm presence of biofilm in chronic wounds
C	Any setting

Inclusion criteria (List criteria such as age groups, study designs, etc. to be included)

[optional]

Adults (18+ years) with Venous leg ulcers (VLU), Diabetic foot ulcers (DFU), Pressure ulcers (PU), Mixed arterial/venous leg ulcers (MAVLU) treated in any setting

Study designs include but are not limited to systematic reviews (CPGs), randomized controlled trials (RCTs) cohort studies, case series, case reports, letters to the editor with relevant data and editorials

These articles must report any clinical signs, symptoms and/or biomarkers, validated or otherwise, thought to be associated with the presence of biofilm in chronic wounds.

Exclusion criteria (List criteria such as study designs, date limits, etc. to be excluded)

[optional]

Patients with wounds resulting from burns, malignant fungating wounds, wounds secondary to conditions such as rheumatoid arthritis or pyoderma gangrenosum

Pay-per-view articles will not be included

Was a search filter applied?

Yes No ✓

If Yes, which one(s), e.g., Cochrane RCT filter, PubMed Clinical Queries filter.

Provide the sources if this is a published filter *[mandatory if YES to previous question]*

N/A

Other notes or comments you feel would be useful to the peer reviewer?

[optional]

Hi Laure,

As this is a scoping review, we have made it pretty sensitive and kept it to our population and the concept we are looking for.

We currently have 5 eligible articles in our possession that turned up serendipitously over the last year or two and this current strategy has captured all of them.

Many thanks for your help with this...

J

Please copy and paste your search strategy below, exactly as run, including the number of hits per line

(Add more space as necessary)

[mandatory]

1. skin ulcer/ or leg ulcer/ or foot ulcer/ or diabetic foot/ or varicose ulcer/ or pressure ulcer/ or wound infection/ or diabetic neuropathies/ or peripheral nervous system diseases/ or wound healing/ or debridement/ or "wounds and injuries"/ or peripheral arterial disease/ or re-epithelialisation/ **266145**
2. Diabetes Mellitus, Type 2/co [Complications]
36725
3. ("peripheral arterial" adj2 disease*).tw.
10441
4. ((chronic or skin or varicose or venous or leg or vascular or foot or diabetic or neuropathic or arterial or isch?emic or neuro-isch?emic or neuroisch?emic or pressure or decubitus or mixed or "mixed ?etiolog*") adj3 (ulcer* or wound*)).tw.
52562

5. ((Preven* or care or heal* or nonheal* or non-heal* or "non heal*" or cicatrix or re-epitheliali* or reepitheliali* or surface* or "lower extremit*" or "lower-extremit*" or debrid* or manag* or bed or "hard to heal" or hard-to-heal or infect*) adj3 (wound* or ulcer*)).tw.

122196

6. (bedsore* or "bed sore*" or bed-sore*).tw.

650

7. ("diabetic foot" adj3 (syndrome or prevention)).tw.

659

8. 1 or 2 or 3 or 4 or 5 or 6 or 7

389861

9. exp Biofilms/

37057

10. biofilm*.tw.

45155

11. 9 or 10

49300

12. 8 and 11

1483

13. Limit 12 to humans

1033

PEER REVIEW ASSESSMENT: THIS SECTION TO BE FILLED IN BY THE REVIEWER

Reviewer:	Email:	Data completed:
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Laure Perrier	l.perrier@utoronto.ca	July 28, 2021
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1. TRANSLATION

A ---No revisions	
B --- Revision(s) suggested	X
C --- Revision(s) required	

If “B” or “C” please provide an explanation or example

Consider removing Line 13: ‘Limit to humans’ and replacing with the following search string:
 13. exp Animals/ NOT (Humans/ and exp Animals/)
 14. 12 NOT 13

This removes all animal studies (reducing risk that a legitimate human study is eliminated) and keeps studies that may combine both animals and humans in the same study (giving the option to abstract human data only).

2. BOOLEAN AND PROXIMITY OPERATORS

A ---No revisions	X
B --- Revision(s) suggested	
C --- Revision(s) required	

If “B” or “C” please provide an explanation or example

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3. SUBJECT HEADINGS

A ---No revisions	X
B --- Revision(s) suggested	
C --- Revision(s) required	

If “B” or “C” please provide an explanation or example

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4. TEXTWORD SEARCHING

A ---No revisions	
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B --- Revision(s) suggested	X
C --- Revision(s) required	

If “B” or “C” please provide an explanation or example

- 1) Line 7: Would consider removing ‘syndrome’ and ‘prevention’ from this search string
 - 2) Consider adding the following text words to ‘wound section’ of search:
 - statis adj3 ulcer*
 - pressure adj3 sore*
 - chronic adj3 wound*
 - chronic adj3 ulcer*
 - diabetic feet
 - 3) For synonyms of ‘biofilm’ – PubMed offers the following:
 - EPS matrix; EPS matrices
 - Extracellular polymeric substance(s)
 - Exopolymers
- *Note that these terms are listed because you have exploded the Subject Heading ‘Biofilms’ -- this is clinical content outside of my expertise so leave you to determine if appropriate to add to search*

5. SPELLING, SYNTAX AND LINE NUMBERS

A ---No revisions	
B --- Revision(s) suggested	X
C --- Revision(s) required	

If “B” or “C” please provide an explanation or example

- 1) It is best to run searches with one term per line as OVID is a great interface but is not overly sophisticated. It doesn’t process everything appropriately once a large set of terms are compiled in one search string.

As an example, when Line 4 has each term entered on an individual line, the results total 61,138 records (see MEDLINE search listed below). Your search above shows 52,562 records when run as one long string on a single line.

Even if you ran your search weeks ago, it is unlikely there would be almost 10,000 new records in a short period of time. Consider cleaving apart all lines with multiple terms (even the lines with multiple Subject Headings) and put one term per line for the entire search (this is recommended for all literature databases). Is more work but ensures you get all your records.

Database: Ovid MEDLINE(R) ALL <1946 to July 27, 2021>

Search Strategy:

- 1 (chronic adj3 ulcer*).tw. (10370)
- 2 (skin adj3 ulcer*).tw. (5668)
- 3 (varicose adj3 ulcer*).tw. (602)
- 4 (venous adj3 ulcer*).tw. (4886)
- 5 (leg adj3 ulcer*).tw. (7373)
- 6 (vascular adj3 ulcer*).tw. (583)
- 7 (foot adj3 ulcer*).tw. (7130)
- 8 (diabetic adj3 ulcer*).tw. (5856)
- 9 (neuropathic adj3 ulcer*).tw. (820)
- 10 (arterial adj3 ulcer*).tw. (577)
- 11 (isch?emic adj3 ulcer*).tw. (1221)
- 12 (neuro-isch?emic adj3 ulcer*).tw. (20)
- 13 (neuroisch?emic adj3 ulcer*).tw. (57)
- 14 (pressure adj3 ulcer*).tw. (8291)
- 15 (decubitus adj3 ulcer*).tw. (1874)
- 16 (mixed adj3 ulcer*).tw. (242)

- 17 ("mixed ?etiolog*" adj3 ulcer*).tw. (13)
- 18 (chronic adj3 wound*).tw. (8039)
- 19 (skin adj3 wound*).tw. (10405)
- 20 (varicose adj3 wound*).tw. (8)
- 21 (venous adj3 wound*).tw. (472)
- 22 (leg adj3 wound*).tw. (646)
- 23 (vascular adj3 wound*).tw. (804)
- 24 (foot adj3 wound*).tw. (1200)
- 25 (diabetic adj3 wound*).tw. (3292)
- 26 (neuropathic adj3 wound*).tw. (62)
- 27 (arterial adj3 wound*).tw. (235)
- 28 (isch?emic adj3 wound*).tw. (490)
- 29 (neuro-isch?emic adj3 wound*).tw. (3)
- 30 (neuroisch?emic adj3 wound*).tw. (13)
- 31 (pressure adj3 wound*).tw. (3478)
- 32 (decubitus adj3 wound*).tw. (73)
- 33 (mixed adj3 wound*).tw. (104)
- 34 ("mixed ?etiolog*" adj3 wound*).tw. (9)
- 35 or/1-34 (61138)

2) OVID doesn't process wildcards at the beginning of a word -- See:
<https://tools.ovid.com/ovidtools/medline.html>

See Lines 17 and Line 34 in search directly above -- it is not capturing the term 'aetiolog*' and you have to search this separately (see below):

- 1 ("mixed etiolog*" adj3 ulcer*).tw. (13)
- 2 ("mixed aetiolog*" adj3 ulcer*).tw. (31)
- 3 ("mixed etiolog*" adj3 wound*).tw. (9)
- 4 ("mixed aetiolog*" adj3 wound*).tw. (7)
- 5 or/1-5 (57)

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6. LIMITS AND FILTERS

A ---No revisions	X
B --- Revision(s) suggested	
C --- Revision(s) required	

If “B” or “C” please provide an explanation or example

You could consider adding a validated age filter (to limit to Adults only) available here by combining the elderly and adult search strings to capture all adult years:
<https://www.jmir.org/2006/4/e25> --

Given your low numbers I wouldn’t add it for this review, but wanted to let you know it exists and is validated:

1. aged/

2.

age*.tw

3. adult.mp

4. Middle Aged/

5. or/1-4

OVERALL

EVALUATION

(Note: If one or more “revision required” is noted above, the below response must be “revisions required”).

A ---No revisions	
B --- Revision(s) suggested	X
C --- Revision(s) required	

Additional comments:

This was done really well – it really reflects your expertise and vast knowledge of the topic area. Overall, would encourage you

to consider one term per line when building a search in the literature databases to ensure you get appropriate results.

Everything else is open for your consideration to include or ignore. Numbers are low so would consider adding as much as possible to ensure your results are comprehensive and search is exhaustive.

Characteristics of included studies.						
Reference	Year of publication	Country of origin	Study design	Setting	Wound aetiology	
Afonso AC, Oliveira D, Saavedra MJ, Borges A, Simoes M. Biofilms in Diabetic Foot Ulcers: Impact, Risk Factors and Control Strategies. <i>International Journal of Molecular Sciences</i> . 2021;22(15):25.	2021	Portugal	Literature review, non-systematic	Not applicable	Diabetic foot ulcers	
Aswathanarayan JB, Rao P, Hm S, Gs S, Rai RV. Biofilm-Associated Infections in Chronic Wounds and Their Management. <i>Advances in experimental medicine and biology</i> . 2023;1370:55-75.	2023	India	Literature review, non-systematic	Not applicable	Chronic wounds (including vascular, diabetic, pressure and burns)	
Bartoszewicz M, Junka A. Biofilm based wound care: strategy for the treatment of chronic wounds affected by the infection caused by microorganisms in the form of biofilms. <i>Leczenie Ran</i> . 2012;9(1):1-6.	2012	Poland	Literature review, non-systematic	Not applicable	Chronic wounds	
Becerra G CC, García A MP, Reyes M YD, Huertas MG. Bacterial biofilms in chronic wounds ; Biopelículas bacterianas em feridas crónicas ; Biopelículas bacterianas en heridas crónicas. <i>Rev Health Forest</i> . 2019;9(1):47 -61.	2019	Colombia	Literature review, non-systematic	Not applicable	Chronic wounds	
Beele H, Van Overschelde P, Olivecrona C, Smet S. A prospective randomized controlled clinical investigation comparing two post-operative wound dressings used	2010	Belgium	Randomized controlled trial	Not reported	Pressure ulcers, Venous leg ulcers	

after elective hip and knee replacement; Mepilex R Border Post-Op versus Aquacel R surgical. International journal of orthopaedic and trauma nursing. 2020;38:100772.							
Bernardo Martin A. Eficacia del cadexómero yodado frente a la hidrofibra de hidrocóloide con plata combinado con desbridamiento cortante en el tratamiento de úlceras por presión con sospecha de biofilm bacteriano :Ensayo clínico con diseño factorial ; Efficacy of iodine cadexomer versus hydrocolloid hydrofibre with silver in pressure ulcers treatment with bacterial biofilm suspicion: Factorial design clinical trial 2017.	2017	Spain	Randomized controlled trial protocol (Thesis)	Multicentre, public, subsidized, and private socio-sanitary centres, urban primary care centres	Pressure ulcers		
Bjarnsholt T, Alhede M, Jensen PO, Nielsen AK, Johansen HK, Homøe P, et al. Antibiofilm properties of acetic acid. Adv Wound Care. 2015;4(7):363-72.	2015	Denmark	Case series + Laboratory	Not reported	Diabetic foot ulcers, Leg ulcers		
Bjarnsholt T, Kirketerp-Møller K, Jensen PO, Madsen KG, Phipps RK, Krogfelt K, et al. Why chronic wounds will not heal: a novel hypothesis. 2008.	2008	Denmark	Literature review, non-systematic (hypothesis paper/perspective article)	Not applicable	Venous leg ulcers, Pressure ulcers, Diabetic foot ulcers		
Bowen G, Richardson N. Biofilm management in chronic wounds and diabetic foot ulcers. Diabetic Foot Journal. 2016;19(4):198-204.	2016	UK	Literature review, non-systematic	Not applicable	Diabetic foot ulcers, Leg ulcers		

Bowlby M, Blume P. Can ultrasound debridement facilitate biofilm removal from diabetic foot ulcers? Podiatry today. 2014;27(8):20 - 9.	2014	USA	Literature review, non-systematic	Not applicable	Diabetic foot ulcers	
Brill FHH, Wodrich M, Pahl S. Biofilms in chronic wounds: Emergence and clinical relevance for wound healing. Vasomed. 2017;29(4):172-3.	2017	Germany	Editorial (summary of a congress presentation)	Not applicable	Chronic wounds	
Brown A. Diagnosing and managing wound infection. Journal of Community Nursing. 2022;36(4):26-34.	2022	UK	Literature review, non-systematic	Not applicable	Chronic, acute, surgical wounds	
Brown A. Dispelling some myths and misconceptions in wound care. Journal of Community Nursing. 2018;32(6):24-32.	2018	UK	Literature review, non-systematic	Not applicable	Wounds	
Castrillón Rivera LE, Palma Ramos A, del Carmen Padilla Desgarenes M. Interferencia de las biopelículas en el proceso de curación de heridas. Dermatología Rev Mex. 2011;55(3):127 - 39.	2011	Mexico	Literature review, non-systematic	Not applicable	Chronic wounds	
Choudhury M, Downie F. A biofilm based wound care pathway in the community setting: a review. Wounds UK. 2022;18(4):14-20.	2022	UK	Literature review, non-systematic	Not applicable	Chronic, non-healing wounds	
Cogo A, Bignozzi AC, Hermans MH, Quint BJ, Snels JP, Schultz G. A desiccation compound as a biofilm- and necrosis-removing agent: a case series. Journal of wound care. 2022;31(10):816-22.	2022	Italy	Case series	Hospital	Venous leg ulcers, Post-traumatic, Vascular ulcers,	

						Ischaemic ulcers, Diabetic foot ulcers
Cooper R. THE ROLE OF BIOFILMS IN CHRONIC FOOT WOUNDS. Podiatry Now. 2016;19(11):30-1.	2016	UK	Literature review, non-systematic	Not applicable	Diabetic foot ulcers	
Cowan T. Biofilms and their management: from concept to clinical reality. Journal of wound care. 2011;20(5):220-6.	2011	UK	Editorial (Report on a lecture)	Not applicable	Chronic wounds	
Cowan T. Prevention and management of wound biofilms: what are the options? A round table discussion. Journal of Wound Care. 2011;20(5):227-30.	2011	UK	Editorial (Report on a round table discussion)	Not applicable	Wounds	
Cowan T. Visible biofilms - a controversial issue! Journal of wound care. 2012;21(3):106.	2012	UK	Editorial	Not applicable	Wounds	
Cowan T. Biofilms and their management: implications for the future of wound care. Journal of wound care. 2010;19(3):117-20.	2010	UK	Editorial (lecture report)	Not applicable	Chronic wounds	
Cutting K, Wolcott R, Dowd S, Chap. Biofilms and Significance to Wound Healing. In: S. P, K. C, editors. Microbiology of Wounds: CRC Press; 2010. p. 233 - 47.	2010	UK	Book Chapter	Not applicable	Wounds	
Darvishi S, Tavakoli S, Kharaziha M, Girault HH, Kaminski CF, Mela I. Advances in the Sensing and Treatment of Wound Biofilms. Angewandte Chemie (International ed in English). 2021.	2021	Switzerland	Literature review, non- systematic	Not applicable	Chronic wounds	

	2017	UK	Case report	Not reported	Venous leg ulcers
Dawkins H. Non-healing venous leg ulcer. <i>British Journal of Nursing</i> . 2017;20:S26-S7.	2020	Brazil	Case series	Outpatient centre caring for patients referred from the municipal health department for diagnosis and treatment in the areas of angiopathy and vascular surgery.	Diabetic foot ulcers, Venous leg ulcers
de Moura MRL, Soares SR, de Azevedo DS, Miranda JS. Treatment protocol for hard-to-heal wounds using a hydrofiber dressing with 1.2% ionic silver, ethylenediaminetetraacetic acid and benzethonium chloride. <i>Journal of Wound Care</i> . 2020;29(10):18-26.					
Dowsett C. Biofilms: A practice-based approach to identification and treatment. <i>Wounds UK</i> . 2013;9(2):68-72.	2013	UK	Literature review, non-systematic (Practice development)	Not applicable	Chronic wounds

Dowsett C, Bellingeri A, Carville K, Garten A, Woo K. A route to more effective infection management: The Infection Management Pathway. Wounds International. 2020;11(3):50-7.	2020	UK	Survey + report of an infection management pathway	Australia, New Zealand, Europe, North America	Diabetic foot ulcers, Venous leg ulcers, Pressure ulcers, Arterial ulcers & Surgical dehiscence wounds
Drago F, Gariazzo L, Cioni M, Trave I, Parodi A. The microbiome and its relevance in complex wounds. Eur J Dermatol. 2019;29(1):6-13.	2019	Italy	Literature review, non-systematic + case series	Hospital ulcer clinic	Pressure ulcers, diabetic foot ulcers
Durbin K. A three-patient evaluation. British journal of nursing (Mark Allen Publishing). 2017;26(Sup20a):S21-S2.	2017	UK	Case series	Acute hospital	Venous leg ulcers, unknown aetiology
Edwards-Jones VAL, Atkin L, Guttormsen K. Biofilm-based wound care: how to cleanse, debride and manage chronic wounds. Wounds UK. 2018;14(4):10-6.	2018	UK	Editorial (expert debate)	Not applicable	Chronic wounds
Gagnon L. Biofilm presents challenge in wound care. Dermatology Times. 2015;36(1):20-.	2015	Canada	Editorial	Not applicable	Wounds
Geng F, Cao S, Bo S, Feng M, Jia Y, Quin J. Effect and cost - effectiveness analysis on using two antibacterial silver dressings on infection of wound with bacterial biofilm. Chinese nursing research. 2022;36(1):44 - 8.	2022	China	Randomized controlled trial	Wound clinic of a tertiary grade hospital	Trauma, Burns, Venous leg ulcers, Surgical wounds,

						Pressure ulcers, diabetic foot ulcers
Gillies A. Surfactants in the treatment of chronic wounds and biofilm management. Journal of Community Nursing. 2019;33(6):34-8.	2019	UK	Literature review, non-systematic	Not applicable	Chronic wounds	Chronic wounds
Gompelman M, van Asten SAV, Peters EJG. Update on the Role of Infection and Biofilms in Wound Healing: Pathophysiology and Treatment. Plastic and reconstructive surgery. 2016;138(3 Suppl):61S-70S.	2016	USA	Literature review, non-systematic	Not applicable	Diabetic foot ulcers	Diabetic foot ulcers
Haemmerle G, Duelli H, Abel M, Strohal R. The wound debrider: A new monofilament fibre technology. British Journal of Nursing. 2011;20(6 SUPPL.):S35-S42.	2011	Austria	Case series	2 hospitals	Diabetic foot ulcers, Venous leg ulcers, Arterial ulcers, Mixed arterial/venous leg ulcers	Diabetic foot ulcers, Venous leg ulcers, Arterial ulcers, Mixed arterial/venous leg ulcers
Haesler E, Ousey K. Evolution of the wound infection continuum. Wounds International. 2018;9(4):6-10.	2018	Australia	Editorial	Not applicable	Wounds	Wounds
Haesler E, Swanson T, Ousey K, Carville K. Clinical indicators of wound infection and biofilm: reaching international consensus. Journal of Wound Care. 2019;28(3):S4-S12.	2019	Australia	eDelphi Survey	Online	Chronic wounds	Chronic wounds
Hampton S. Managing biofilm by using dressings. British journal of community nursing. 2015;Community Wound Care:S10, S2.	2015	UK	Editorial (Clinical comment)	Not applicable	Chronic wounds	Chronic wounds

Hernández Ortiz JÁ, Navarro Fernández AM, Galera Barreiro AM, Marín Bello MJ, Cazallilla Pérez A, Lopes Pereira AM. Abordaje de una herida por roce o fricción de categoría III, con presencia de biofilm, tras contención mecánica. Gerokomos. 2018;29(2):105-7.	2018	Spain	Case report	Intensive care unit	Pressure ulcers
Hsiao-Chen L. Using aquacel™ ag+ dressing in treating poor healing chronic wound after surgical debridement - Case report and literature review. Journal of Wound Care. 2020;29(SUPPL 7B):217.	2020	Taiwan	Case report	Surgical department	Chronic wounds
Hurlow J, Bowler PG. Potential implications of biofilm in chronic wounds: a case series. Journal of wound care. 2012;21(3):109-10, 12, 14 passim.	2012	USA	Case series	Acute and outpatient	Venous leg ulcers, Leg ulcers, Pressure ulcers, Mixed Arterial Venous leg ulcers, traumatic, oedema, burns
Hurlow J, Bowler PG. Clinical experience with wound biofilm and management: A case series. Ostomy Wound Management. 2009;55(4):38-49.	2009	USA	Case series	Not reported	Venous leg ulcers, Ischaemic ulcers
Hurlow J. Understanding biofilm: what a community nurse should know. British journal of community nursing. 2016;21:S26-S33.	2016	UK	Literature review, non-systematic	Not applicable	Chronic wounds, Venous leg ulcers
Hurlow J, Blanz E, Gaddy JA. Clinical investigation of biofilm in non-healing wounds by high resolution microscopy techniques. Journal of wound care. 2016;25:S11-S22.	2016	USA	Laboratory study	Wound care centre	Leg ulcers, Venous leg ulcers, traumatic, Ischaemic ulcers,

Johani K, Malone M, Jensen SO, Gosbell IB, Dickson HG, Hu H, et al. Microscopy visualisation confirms multi-species biofilms are ubiquitous in diabetic foot ulcers. 2017.	2017	Australia	Laboratory study	High risk foot service	Diabetic foot ulcers
Kataoka Y, Kunitatsu M, Nakagami G, Koudounas S, Weller CD, Sanada H. Effectiveness of ultrasonic debridement on reduction of bacteria and biofilm in patients with chronic wounds: A scoping review. International Wound Journal. 2021;18(2):176-86.	2020	Japan	Scoping review	Not applicable	Venous leg ulcers
Keast D, Swanson T. Clinical innovation: 2016 wound infection consensus document. Wounds International. 2017;8(2):19-21.	2017	Australia	Editorial	Not applicable	Wounds
Swanson T, Keast D, Cooper R, Black J, Angel D, Schultz G, et al. Ten top tips: identification of wound infection in a chronic wound. Wounds International. 2015;6(2):22-7.	2014	Canada	Literature review, non-systematic	Not applicable	Chronic wounds
Leaper DJ, Schultz G, Carville K, Fletcher J, Swanson T, Drake R. Extending the TIME concept: What have we learned in the past 10 years? International Wound Journal. 2012;9(SUPPL. 2):1-19.	2012	UK	Literature review, non-systematic	Not applicable	Chronic wounds
Lenselink E, Andriessen A. A cohort study on the efficacy of a polyhexanide-containing biocellulose dressing in the treatment of biofilms in wounds. Journal of Wound Care. 2011;20(11):534-9.	2011	Netherlands	Cohort	Outpatient wound clinic	Venous leg ulcers, Mixed arterial/venous leg ulcers, Surgical wounds, Burns,

						Arterial ulcers, Diabetic foot ulcers
Lloyd Jones M. Series 3. Wound infection 3.1: the wound infection continuum explained. <i>British Journal of Healthcare Assistants</i> . 2018;12(5):218-20.	2018	UK	Editorial (review of a best practice statement)	Not applicable	Wounds	
Malone M, Schultz G: Challenges in the diagnosis and management of wound infection. <i>The British journal of dermatology</i> . 2022;187(2):159-66.	2022	Australia	Literature review, non-systematic	Not applicable	Multiple aetiologies including but not limited to diabetes-related foot ulcers, venous leg ulcers, surgical wounds, and burns.	
Malone M, Schwarzer S, Radzieta M, Jeffries TT, Walsh A, Dickson HG, et al. Effect on total microbial load and community composition with two vs six-week topical Cadexomer Iodine for treating chronic biofilm infections in diabetic foot ulcers. <i>International Wound Journal</i> . 2019;16(6):1477 - 86.	2019	Australia	Randomized controlled trial	Acute tertiary referral hospital	Diabetic foot ulcer	
McGuire J, D'Alessandro J. Combating Biofilms In The Chronic Wound. <i>Podiatry Today</i> . 2016;29(8):32-40.	2016	USA	Literature review, non-systematic	Not applicable	Chronic wounds	
McGuire J, Love E, Vlahovic TC, Khan K, Labbad ZG, Robinson L, et al. The ABCESS System for Chronic Wound Management: A New Acronym for Lower Extremity Wound	2020	USA	Literature review, non-systematic	Not applicable	Chronic wounds/lower extremity wounds	

Management. Wounds : a compendium of clinical research and practice. 2020;32:S1-S25.				(Acronym proposal)			
Mendoza RA, Hsieh J-C, Galiano RD. The Impact of Biofilm Formation on Wound Healing. In: Hakan Dokan K, editor. Wound healing - Current perspectives: IntechOpen; 2019. p. 235 - 50.	2019	USA		Book chapter	Not applicable	Chronic wounds	
Metcalf D, Bowler PG. Perceptions of Wound Biofilm by Wound Care Clinicians. Wounds-Compend Clin Res Pract. 2019;31(3):E14-E7.	2019	UK		Survey	Online (UK, Germany, USA, Italy)	Non-healing wounds	
Metcalf DG, Bowler PG. Biofilm delays wound healing: A review of the evidence. Burns Trauma. 2013;1(1):5-12.	2013	UK		Literature review, non-systematic	Not applicable	Chronic wounds, Venous leg ulcers, Diabetic foot ulcer + Cellulitis, Peripheral arterial ulcers, highly exuding ulcer	
Metcalf DG, Bowler PG. Clinician perceptions of wound biofilm. International wound journal. 2016;13(5):717-25.	2016	UK		Survey	Online (UK, USA, Germany, Italy)	Chronic/recalcitrant wounds	
Metcalf D, Parsons D, Bowler IP. DEVELOPMENT OF A NEXT-GENERATION ANTIMICROBIAL WOUND DRESSING. Acta medica Croatica : časopis Hrvatske akademije medicinskih znanosti. 2016;70(1):49-56.	2016	UK		Literature review, non-systematic	Not applicable	Chronic wounds	

				(Clinical observation)			
Metcalf DG, Bowler PG, Hurlow J. A clinical algorithm for wound biofilm identification. Journal of Wound Care. 2014;23(3):137-42.	2014	UK		Literature review, non-systematic (Algorithm proposal)	Not applicable	Chronic wounds	
Metcalf DG, Parsons D, Bowler PG. Clinical safety and effectiveness evaluation of a new antimicrobial wound dressing designed to manage exudate, infection and biofilm. International Wound Journal. 2017;14(1):203-13.	2017	UK		Case series	60 health care facilities (hospitals, clinics, nursing homes) and community settings	Venous leg ulcers, Mixed Arterial/Venous leg ulcers, Arterial ulcers, Pressure ulcers, Diabetic foot ulcers, Leg ulcers, Traumatic ulcers, Cyst, Other	
Metcalf D, Parsons D, Bowler P. A next-generation antimicrobial wound dressing: a real-life clinical evaluation in the UK and Ireland. Journal of wound care. 2016;25(3):132, 4-8.	2016	UK		Case series	8 healthcare facilities	Mixed arterial, venous leg ulcers, Venous leg ulcers, Diabetic foot ulcers, Pressure ulcers, Arterial ulcers, Leg ulcers, traumatic, cyst, other	

Miller CC, Miller MK, Ghaffari A, Kunimoto B. Treatment of chronic nonhealing leg ulceration with gaseous nitric oxide: A case study. <i>Journal of Cutaneous Medicine and Surgery</i> . 2004;8(4):233-8.	2004	Canada	Case report	Community/home	Venous leg ulcers
Min W. Antibacterial protease combined with silver dressing for the intervention of bacterial biofilm infection in chronic wounds. https://trialsearch.who.int/Trial2.aspx?TrialID=ChiCTR1800019687 . 2018.	2018	China	Randomized controlled trial protocol	Hospital	Chronic wounds
Morris C. Why biofilm-based wound management is vital. <i>Journal of Community Nursing</i> . 2020;34(5):8-9.	2020	UK	Editorial	Not applicable	Chronic wounds
Murphy C, Atkin L, Vega de Ceniga M, Weir D, Swanson T, Walker A, et al. Embedding Wound Hygiene into a proactive wound healing strategy. <i>Journal of Wound Care</i> . 2022;31(Sup4a):S1-S19.	2022	Canada	Consensus document	Not applicable	Hard to heal wounds
Oldfield R. Exuding venous leg ulcer with signs of biofilm. <i>British journal of nursing</i> (Mark Allen Publishing). 2017;26(Sup20a):S24-S5.	2017	UK	Case report	Not reported	Venous leg ulcers
Palomar Llatas F, Pastor Orduña MI, Bonías López J, Fornes Pujalte B, Sierra Talamantes C, Zamora Ortiz J, et al. Características y manejo del lecho de las heridas crónicas ; Characteristics and management of the bed of chronic wounds. <i>III Dermatol</i> . 2018;12(33):10 - 8.	2018	Spain	Literature review, non-systematic	Not applicable	Chronic wounds
Pankhurst S. Heavily exuding venous leg ulcer with suspected biofilm. <i>British journal of nursing</i> (Mark Allen Publishing). 2017;26(Sup20a):S29-S30.	2017	UK	Case report	Leg ulcer clinic	Venous leg ulcers

Pedro I, Saraiva S. INTERVENÇÕES DE ENFERMAGEM NA GESTÃO DE BIOFILMES EM FERIDAS COMPLEXAS. Journal of aging and innovation. 2012;1(6):78 - 88.	2012	Portugal	Systematic review	Not applicable	Chronic/complex wounds
Percival SL, Hill KE, Malic S, Thomas DW, Williams DW. Antimicrobial tolerance and the significance of persister cells in recalcitrant chronic wound biofilms. Wound Repair Regen. 2011;19(1):1-9.	2011	UK	Literature review, non-systematic	Not applicable	Chronic wounds
Percival SL, Hill KE, Williams DW, Hooper SJ, Thomas DW, Costerton JW. A review of the scientific evidence for biofilms in wounds. Wound Repair Regen. 2012;20(5):647-57.	2012	UK	Literature review, non-systematic	Not applicable	Chronic wounds, Diabetic foot ulcers
Percival SL, Vuotto C, Donelli G, Lipsky BA. Biofilms and wounds: An identification algorithm and potential treatment options. Adv Wound Care. 2015;4(7):389-97.	2015	UK	Literature review, non-systematic (algorithm proposal)	Not applicable	Chronic wounds
Phillips PL, Wolcott RD, Fletcher J, Schultz GS. Biofilms made easy. International Wound Journal. 2010;1(3).	2010	USA	Literature review, non-systematic	Not applicable	Chronic wounds
Pinto GPNM. Biofilmes e feridas crônicas 2016.	2016	Portugal	Systematic review (master's thesis, algorithm proposal)	Not applicable	Chronic wounds

Carlos Poblete J, Juliany Lino Gomes S, Flávia Cristina Z, Thais R, Maria Helena Melo L. Biofilme e feridas crônicas: reflexões para o cuidado de enfermagem. Current Nursing Journal. 2017;81.	2017	Brazil	Integrative review	Not applicable	Chronic wounds
Price J, Boulton Z. Case 13: chronic painful ulcer on the heel of a diabetic foot. Journal of wound care. 2016;25(3):S21.	2016	UK	Case report	Not applicable	Pressure ulcer on diabetic foot
Pupp G, Koivunen R. How To Assess The Bacterial Burden Of DFUs. Podiatry Today. 2012;25(7):62-8.	2012	USA	Literature review, non-systematic	Not applicable	Diabetic foot ulcers
Rama D, Fonseca B, M. B. 1a RECOMENDAÇÃO BRASILEIRA PARA O GERENCIAMENTO DE BIOFILME EM FERIDAS CRÔNICAS E COMPLEXAS2018.	2018	Brazil	Practice recommendation	Not applicable	Complex/chronic wounds
Rodgers A, Watret L. How might biofilms affect dressing choice for the diabetic foot? Diabetic Foot Journal. 2008;11(2):86-.	2008	UK	Editorial	Not applicable	Diabetic foot ulcers
Roes C, Calladine L, Morris C. Biofilm management using monofilament fibre debridement technology: outcomes and clinician and patient satisfaction. Journal of wound care. 2019;28(9):608-22.	2019	Germany	Case series	Real world routine care settings across all regions of the UK + web-based survey	Leg ulcers, Pressure ulcers, Delusced surgical ulcer, other

Salazar Trujillo MA. Management of complex wounds treatment with a hydrofiber dressing in a public hospital in Bogota (Colombia) ; Лечение сложных ран гидроволоконистыми перевязочными средствами в условиях городской больницы Боготы (Колумбия). Wounds and wound infections. 2021;8(3):26 - 34.	2021	Colombia	Case series	Dept. of plastic surgery	Vascular ulcers, post-traumatic ulcers, Diabetic foot ulcers, Pressure ulcers, post-surgical oncological ulcers, Arterial ulcers, post-surgical ulcers
Salazar Trujillo MA, Ortiz Rodriguez JE, Ospina AZ. Efectividad de un apósito de hidrofibra reforzada, con plata iónica al 1,2%, potenciado con EDTA y cloruro de bencetonio: casos de estudio. Journal of Wound Care. 2020;29(Lat AM Sup1):1-12.	2020	Colombia	Case series	Wound clinic	Venous leg ulcers, Pressure ulcers, Arterial ulcers, Surgical site infections, Friction wounds, burns, facial (retinoblastoma), mastitis, traumatic, Seroma, fasciotomy
Sanjuan Sanchez MJ. REVISIÓN SISTEMÁTICA. ESTRATEGIAS TERAPÉUTICAS PARA LA GESTIÓN DEL BIOFILM BACTERIANO EN HERIDAS CRÓNICAS. Alicante: Universidad de Alicante; 2018.	2018	Spain	Systematic review (thesis)	Not applicable	Chronic wounds

Santos V, Santos AS, Menoita E. A ABORDAGEM DE BIOFILMES EM FERIDAS: ESTUDOS DE CASO. Journal of aging and innovation. 2013;2(1):76 - 96.	2013	Portugal	Case series	NR	Venous leg ulcers, Diabetic foot ulcers, Pressure ulcers
Schultz G, Bjarnsholt T, James GA, Leaper DJ, McBain AJ, Malone M, et al. Consensus guidelines for the identification and treatment of biofilms in chronic nonhealing wounds. Wound repair and regeneration : official publication of the Wound Healing Society [and] the European Tissue Repair Society. 2017;25(5):744-57.	2017	USA	Consensus guideline	Not applicable	Chronic wounds
Siaw-Sakyi V. Identification and management of biofilm in chronic wounds. Journal of Community Nursing. 2018;32(5):32-40.	2018	UK	Literature review, non-systematic	Not applicable	Chronic wounds
St-Cyr D. [Biofilms. What are they? Where are they? What impact do they have on wound care?]. Perspective infirmière : revue officielle de l'Ordre des infirmières et infirmiers du Québec. 2011;8(3):36-8.	2011	Canada	Literature review, non-systematic	Not applicable	Chronic wounds
Swanson T. Innovations in the assessment and diagnosis of wound infection. Wounds International. 2011;2(1):5-6.	2011	Australia	Literature review, non-systematic	Not applicable	Chronic wounds
Swanson T, Ousey K, Haesler E, Bjarnsholt T, Carville K, Idensohn P, et al. Wound Infection in Clinical Practice: Principles of Best Practice: International Consensus Update: Wounds International; 2022.	2022	Australia	Consensus document	Not applicable	Chronic wounds

Swanson T, Keast D, Bain K, Bain M. Preventing and treating infection in wounds: translating evidence and recommendations into practice. Wounds International. 2020;11(4):82-7.	2020	Australia	Survey	Virtual, face to face, 19 countries	Chronic wounds
Swanson T, Haesler E, Angel D, Sussman G. IWII Wound infection in clinical practice consensus document 2016 update. Wound Practice & Research. 2016;24(4):194-8.	2016	Australia	Editorial (Report on a consensus document update)	Not applicable	Wound infection (various aetiologies)
Swanson T. Understanding biofilm in practice: a global survey of health professionals. Journal of Wound Care. 2017;26(8):426-40.	2017	Australia	Survey	Global(UK, other, Europe, Australia, New Zealand, USA, Canada, Japan, Middle East, Asia, South America	Wounds
Temprano-Andrés SA, Martínez-Antón S. Aproximación a la importancia del biofilm en las heridas crónicas. Revisión bibliográfica. Enfermería Dermatológica. 2020;14(39):23-8.	2020	Spain	Systematic review	Not applicable	Chronic wounds
Torrósga Jerez L. MÉTODOS DE DIAGNÓSTICO PARA LA IDENTIFICACIÓN DE BIOFILM EN HERIDAS CRÓNICAS. REVISIÓN SISTEMÁTICA. Alicante: Universidad de Alicante; 2015.	2015	Spain	Systematic review (thesis)	Not applicable	Chronic wounds

Anonymous. Biofilm as the Cause of Non-Healing Wounds. Medsurg nursing : official journal of the Academy of Medical-Surgical Nurses. 2015;24(2):Suppl-3.	2015	USA	Editorial	Not applicable	Non-healing wounds
Vallejo A, Wallis M, McMillan D, Horton E. Use of low-frequency contact ultrasonic debridement with and without polyhexamethylene biguanide in hard-to-heal leg ulcers: an RCT protocol. Journal of Wound Care. 2021;30(5):372-9.	2021/2022	Australia	Randomized controlled trial + Protocol	Community specialised wound clinic	Venous leg ulcers, arterial ulcers, Mixed arterial/venous leg ulcers
Vallejo A, Wallis M, McMillan D. Use of low-frequency contact ultrasonic debridement with and without polyhexamethylene biguanide in hard-to-heal leg ulcers: an RCT. J Wound Care. 2022;31(8):670-81.					
Vallejo A, Wallis M, Horton E, McMillan DJ. Low-frequency ultrasonic debridement and topical antimicrobial solution Polyhexamethylene biguanide for use in chronic wounds: a case series. Wound Practice & Research. 2018;26(1):4 - 13.	2018	Australia	Case series	Not reported	Venous leg ulcers
Vyas KS, Wong LK. Detection of Biofilm in Wounds as an Early Indicator for Risk for Tissue Infection and Wound Chronicity. Annals of plastic surgery. 2016;76(1):127-31.	2016	USA	Literature review, non-systematic	Not applicable	Chronic wounds
Walker M, Metcalf D, Parsons D, Bowler P. A real-life clinical evaluation of a next-generation antimicrobial dressing on acute and chronic wounds. Journal of wound care. 2015;24(1):11-22.	2015	UK	Case series	33 Healthcare facilities	Venous leg ulcers, Traumatic ulcers, Pressure ulcers, Abscess, Cellulitis, Inflammation,

							Radiation, Rectal, Tumour
Webb R. A chronic case of confusion. <i>Journal of Wound Care</i> . 2017;26(8):421-.	2017	UK	Editorial	Not applicable	Chronic wounds		
Wei D, Zhu XM, Chen YY, Li XY, Chen YP, Liu HY, et al. Chronic wound biofilms: diagnosis and therapeutic strategies. <i>Chinese Medical Journal</i> . 2019;132(22):2737-44.	2019	China	Systematic review	Not applicable	Chronic wounds		
Westgate S, Cutting K. The role of microbial biofilms in chronic and acute wounds. <i>Nursing & Residential Care</i> . 2011;13(11):518-21.	2011	UK	Literature review, non-systematic	Not applicable	Chronic and acute wounds		
White RJ, Cutting KF. Wound biofilms-are they visible? <i>Journal of wound care</i> . 2012;21(3):140-1.	2012	UK	Letter	Not applicable	Chronic wounds		
White R, Cooper R, Edwards-Jones V. What is the role of biofilms in wound healing? <i>Wounds UK</i> . 2012;8(2):20-4.	2012	UK	Editorial (Debate report)	Not applicable	Wounds		
Widgerow AD. Persistence of the chronic wound - implicating biofilm. <i>Wound Healing Southern Africa</i> . 2008;1(2):05 - 7.	2008	S. Africa	Literature review, non-systematic	Not applicable	Chronic wounds		
Wilson P, Gillen C, Hughes M. A clinical case series on the effectiveness of an enhanced ionic silver hydrofiber dressing in the management of diabetic foot ulceration. <i>Diabetic Foot Journal</i> . 2018;21(4):239-46.	2018	Ireland	Case series	Podiatry-led foot clinic in a large urban	Diabetic foot ulcers		

				tertiary referral centre	
Wolcott RD, Rhoads DD, Bennett ME, Wolcott BM, Gogokhia L, Costerton JW, et al. Chronic wounds and the medical biofilm paradigm. Journal of wound care. 2010;19(2):45-6, 8-4650, 52-53.	2010	USA	Literature review, non-systematic + Case series	Not reported	Venous leg ulcers, Traumatic (PAD)
Bjarnsholt T, Cooper R, Fletcher J, K. K-M, Malone M, Schultz G, et al. World Union of Wound Healing Societies (WUWHS), Florence Congress, Position Document. Management of biofilm. Wounds International [Internet]. 2016.	2016	Denmark	Position document	Not applicable	Hard-to-heal wounds
Young L. Identifying infection in chronic wounds. Wound Practice & Research. 2012;20(1):38-44.	2012	Australia	Literature review, non-systematic	Not applicable	Chronic wounds

Table of excluded full text reports (n = 180)	
Reference	Reason for exclusion
Ammons MCB. Fuchs AL. Tripet BP. et al. Chronic diabetic wounds: Longitudinal profiling of the evolving microbiome and metabolic landscape in diabetic patients. FASEB Journal. 2017; https://doi.org/10.1096/fasebj.31.1_supplement.944.9	Irretrievable
Bello YM. Falabella AF. & Cazzaniga AL. Are biofilm present in human chronic wounds? Presented at the symposium on advanced wound care and medical research forum on wound repair in Las Vegas, NV, April 30-May 3 2001	Irretrievable
Brambilla R. Simone T. Giuseppina G. et al. Local control of bioburden and infection in mdrb. J Wound Care. 2020; 29: 276	Irretrievable
Cheng H. & Huang G. Research progress on bacterial biofilms in chronic wounds. Chinese Nursing Research. 2020; 34(10): 1754-1758	Irretrievable
Chrysostomou D. Biofilm-comparison of two methods of wound swabbing. J Wound Care. 2017; 26: 185	Irretrievable
Cogo A. Quint BJ. & Bignozzi CA. The clinical efficacy of a novel desiccant agent, debrichem in the treatment of chronic skin wounds: A case series of 50 patients . J WOUND CARE. 2020; 29: 63-64	Irretrievable
Colella R. New biotechnologies: the importance of biofilm management and wound bed preparation to promote tissue repair in the management of the diabetic foot. Case report. Italian Journal of Medicine. 2021; 15(3): 26	Irretrievable
Liu J & Liu Y. Research advances on the formation mechanism and diagnosis of bacteria biofilms in chronic wounds. Chinese Journal of burns. 2021; 37(7): 692-696	Irretrievable
Nakagami G. Evaluation of wound management based on biofilm detection at bedside: A randomized controlled trial. JPRN-UMIN000029684. 2017 (Accessed 5 September 2022)	Irretrievable
Rastelli C. Nedjua B. Isaac CGJ. Et al. The use of Co2 Laser for the debridement of hard to heal wounds. J WOUND CARE. 2020; 29(Suppl 7B): 173	Irretrievable
Rhoads D. Wolcott R. Cutting KF et al. (2007) Biofilms and management in chronic infected wounds. In McBain A. Allison D. Pratten J. et al. eds. Biofilms: coming of age. Cardiff, Bioline.	Irretrievable

Suleman L. & Chapp L. Biofilm -infected pressure ulcers: Current knowledge and emerging treatment strategies. Reference details unknown.	Irretrievable
Swanson T. Wound infection and management: Playing detective with each dressing change. J WOUND CARE. 2020; 29: 118	Irretrievable
Thomas JG. Corum L & Mantlagh H. Chronic wounds: infectious diseases that won't go away. Wounds, gauzes and biofilms combine for an ugly triad. Advance for Administrators of the Laboratory . 2012; 21(5): 18-20	Irretrievable
Unknown author. ¿Tenemos las herramientas necesarias para luchar contra el biofilm? (Do we have the necessary tools to fight biofilms)? Journal unknown; 32(1): 14-5	Irretrievable
Unknown author. Biofilm and slough. Wounds UK. 2010; 6(1): 162-163	Irretrievable
Unknown author. Biofilms and the role of debridement in chronic wounds. Wounds UK. 2010; 6(1): 160-162	Irretrievable
Wolcott R. Dowd S. Kennedy J. et al. Biofilm based wound care. Advances in Wound Care. 2010; 1(3): 311-318	Irretrievable
Wound Care Center. Biofilm based wound management. Available at www.woundcarecenter.net/CHAPTERTEXT.pdf (2006)	Irretrievable
Yu JA. & Gao XX. Bacterial biofilm and chronic wound infection. Chinese Journal of Burns. 2019; 35(12): 842-847	Irretrievable
Amaral V. Silva NDS. Osis SL. Treatment of wound after amputation toes of the left foot in patients with diabetes mellitus. J WOUND CARE. 2017; 26 (suppl 6): 312	Wrong population
Serralta VW. Harrison-Balestra C. Cazzinaga AL. et al. Lifestyles of bacteria in wounds: Presence of biofilm? Wounds. 2001; 13: 29-34	Wrong population
Heridas Crónicas: Biofilm y la importancia del Desbridamiento (Biofilm and the importance of debridement). Sociedad Iberoamericana de Información Científica (SIIC) Buenos Aires. Biofilm y la Importancia del Desbridamiento	No concept data
Alkhatieb MT. Biofilm in the diabetic foot: an added challenge. Gerokomos. 2019; 30(3): 157 - 160	No concept data
Amin O. Wright BJ. Schultz G. et al. Microbial biofilms and chronic wounds. Microorganisms. 2017; 5(9):	No concept data
Ammons MCB. Anti-biofilm strategies and the need for innovation in wound care. Recent Patents on Anti-infective Drug Discovery. 2010; 5(1): 10 -17	No concept data

Armstrong DG. Edmonds ME. & Serena TE. Point-of-care fluorescence imaging reveals extent of bacterial load in diabetic foot ulcers. International Wound Journal. 2023; 20(2): 554 -566	No concept data
Ascenzioni F. Cloeckaert A. Di Domenico EG. et al. Editorial: Microbial biofilms in chronic and recurrent infections. Frontiers in Microbiology. 2021; 12: 1-3	No concept data
Attinger C. & Wolcott R. Clinically addressing biofilm in chronic wounds. Advances in Wound Care. 2012; 1(3): 127-132	No concept data
Ayush R. Ankit R. Singh S. et al. Cadexomer versus povidone iodine dressing in chronic leg ulcers- a prospective study. Student's Journal of Health Research. 2023; 4(3):	No concept data
Banu A. Noorul Hassan MM. Rajkumar J. et al. Spectrum of bacterial associated with diabetic foot ulcer and biofilm formation: A prospective study. Australasian Medical Journal. 2015; 8(9): 280-285	No concept data
Barbara DA. Fabrizio O. Morais D'Autilio Margarida FL. et al. Infection's control and regenerative boost with silver sulfadiazine. J WOUND CARE. 2020; 29: 303	No concept data
Barker JC. Khansa I. & Gorddillo GM. A formidable foe is sabotaging your results: what you should know about biofilms and wound healing. Plast Reconstr Surg. 2017; 139(5): 1184e - 1194e	No concept data
Bartoszewicz M. Junka A. Dydak K. et al. OCENA SKUTECZNOŚCI OPATRUNKU URGOCLEAN® AG WZGLĘDEM FORM BIOFILMOWYCH PATOGENÓW RAN PRZEWLEKŁYCH (Evaluation of the efficacy of Urgoclean®AG dressing against biofilm forms of chronic wound pathogens). Leczenie Ran. 2018; 15(2): 45-50	No concept data
Benavent E. Soldevila L. & Murilo O. Protocolo diagnóstico de las infecciones de úlceras del pie diabético (Diagnostic protocol for diabetic foot ulcer infections). Medicine. 2018; 12(51): 3048-51	No concept data
Bertesteanu S. Triaridis S. Stankovic M. et al. Polymicrobial wound infections: Pathophysiology and current therapeutic approaches. International journal of pharmaceutics. 2014; 463: 119-126	No concept data
Bianchi T. Wolcott RD. Peghetti A. et al. Recommendations for the management of biofilm: A consensus document. 2016; 0(0): 1 - 11	No concept data
Bianchi T. Peghetti A. & Pomponio G. Biofilm: It is time for consensus. J WOUND CARE. 2015; 24(7): 291	No concept data
Bjarnsholt T. The role of bacterial biofilms in chronic infections. Acta Pathologica Microbiologica et Immunologica Scandinavica. 2013; 0(136): 1-51	No concept data

Black CE. & Costerton JW. Current concepts regarding the effect of wound microbial ecology and biofilms on wound healing. <i>Surg Clin N Am.</i> 2010; 90: 1147-1160	No concept data
Boutli-Kaspidou F. Delli F. Avogoustinaki N. et al. What are biofilms? Evaluation and management in open skin wounds. <i>JEADV.</i> 2006; 20: 735-767	No concept data
Brantley J. Park H. Sanchez PJ. et al. The use of a novel antimicrobial and purified native collagen matrix combination to manage bioburden and support healing in challenging wounds: A clinical evaluation. <i>Wounds International.</i> 2016; 7(3): 40-45	No concept data
Brinkert D. Ali M. Naud M. et al. Negative pressure wound therapy with saline instillation: 131 patient case series. <i>International Wound Journal.</i> 2013; 10: 56-60	No concept data
Brognara L. Salmaso L. Mazzotti A. et al. Effects of probiotics in the management of infected chronic wounds: From cell culture to human studies. <i>Curr Clin Pharmacol.</i> 2020; 15(3): 193-206	No concept data
Brown A. & Yorke M. Drawtex: Breaking the vicious circle of cellular and molecular imbalances. <i>Br J Community Nurs.</i> 2013: S42-S49	No concept data
Caldas Arias L. Bacterias-biofilms y resistencia antimicrobiana (Bacterial biofilms and antimicrobial resistance). <i>Revista Facultad Ciencias de la Salud. Universidad del Cauca.</i> 2015; 17(1): 20-27.	No concept data
Carpenter S. Davis S. Fitzgerald R. et al. Expert recommendations for optimizing outcomes in the management of biofilm to promote healing of chronic wounds. <i>Wounds.</i> 2016; 28(Suppl 6): S1-S20	No concept data
Carter MJ. & Myntti MF. Cost-utility of a biofilm-disrupting gel versus standard of care in chronic wounds: A Markov microsimulation model based on a randomised controlled trial. <i>J WOUND CARE.</i> 2022; 28(7 N American suppl): S24-S39	No concept data
Carville K. Howse L. Edmonson M. et al. Nurse practitioners and their use of low-frequency ultrasound debridement in the management of chronic wounds. <i>Wounds Practice and Research.</i> 2018; 26(3): 122-126	No concept data
Chang YJR. Perry J. & Cross K. Low-Frequency ultrasound debridement in chronic wound healing: A systematic review of current evidence. <i>Plastic Surgery.</i> 2017; 25(1): 21-26	No concept data
Chen V. Burgess JL. Verpile R. et al. Novel diagnostic technologies and therapeutic approaches targeting chronic wound biofilms and microbiota. <i>Curr Dermatol Rep.</i> 2023; 11(2): 60-72	No concept data

Cogo A. Bignozzi CA. & Quint B. The clinical efficacy of a novel desiccant agent, debrichem in the treatment of chronic skin wounds. A case series of 50 patients. J WOUND CARE. 2020; 29: 63-64	No concept data
Cole W. Human acellular dermal matrix paired with silver-zinc coupled electroceutical dressing results in rapid healing of complicated diabetic wounds of mixed etiology: a novel case series. Wounds: A Compendium of Clinical Research & Practice. 2016; 28(7): 241-7	No concept data
Cooper R. Biofilms and wounds: Much ado about nothing? Wounds UK. 2010; 6(4): 84 - 90	No concept data
Cooper R. Bjarnsholt T. & Alhede M. Biofilms in wounds: a review of present knowledge. J WOUND CARE. 2022; 23(11): 570 - 582	No concept data
Cooper R. Chapter 6: Understanding biofilms. In Essential Wound Microbiology, Edwards-Jones V. (ed). Oxford University Press, Oxford, 2016	No concept data
Crew JR. Varilla R. Rocas A. et al. Neutrophase™ with Sorbact® dramatically enhances the speed of wound healing. Wound Repair & Regeneration. 2011; 19(2): A19	No concept data
Cutting KC. & Percival SL. Biofilm management. Nurs Standard. 2009; 36: 389-95	No concept data
Cutting KF. Why use topical antiseptics? J WOUND CARE. 2011; 20(Suppl 2):	No concept data
Da Conceição Mota M. Cabral de Melo S. & Pereira Costa T. Estrategia de gestao de biofiles em feridas cronicas: uma revisao dea literatura (Biofilm management strategy in chronic wounds: a literature review). J. Tissue Regeneration & Healing. 2012: 10-18	No concept data
DesJardins H. Char S. Marasco P. et al. Efficacy of hydromechanical therapy in nonhealing, chronic wounds as a cost and clinically effective wound care modality. Wounds: A Compendium of Clinical Research & Practice. 2021; 33(11): 296-303	No concept data
Ding X. Tang Q. Xu Z. et al. Challenges and innovations in treating chronic and acute wound infections: from basic science to clinical practice. Burns & Trauma. 2022; 10: tkac014	No concept data
Dinh T. & Lunborg M. Assessing the impact of biofilm and the microbiome in diagnosing and treating DFUs. Podiatry today. 2016; 29(8): 16-20	No concept data
Esquivia Castillo L. Validez y fiabilidad de los instrumentos para la valoración clínica de infección en heridas crónicas: una revisión sistemática (Validity and reliability of instruments for the clinical	No concept data

assessment of infection in chronic wounds: a systematic review), in Facultad de Ciencias de la Salud. 2023, Universidad de Alicante: Alicante	
Evelhoch SR. Biofilm and chronic non healing wound infections. The Surgical Clinics of North America. 2020; 100(4): 727-732	No concept data
Fazli M. Bjarnsholt T. Kirketerp-Moller K. et al. Non-Random distribution of Pseudomonas aeruginosa and Staphylococcus aureus in chronic wounds. J Clin Microbiol. 2009;47(12): 4084 - 4089	No concept data
Formosa C. & Vella L. Characteristics predicting the outcome in individuals with diabetic foot ulcerations. Diabetic Medicine. 2016; 33: 52	No concept data
Gajula B. Munnamgi S. & Basu S. How bacterial biofilms affect chronic wound healing: a narrative review. International Journal of Surgery: Global Health. 2020; 3(2): e16	No concept data
Goss SG. Alcantara SD. Gendics C. et al. Sharp debridement reduces biofilm more robustly than planktonic bacteria in chronic venous leg ulcers. Wound Repair & Regeneration. 2014; 22(2): A42	No concept data
Greener M. UrgoClean Ag: evidence base and mode of action. British Journal of Nursing. 2017; 26: S12-S15	No concept data
Håkans E. Sårvård med fokus på sekret och biofilm : en litteraturstudie (Wound care with a focus on secretions and biofilm: a literature study). Yrkeshögskolan Novia, 2018	No concept data
Hall MR. McGillicuddy E. & Kaplan LJ. Biofilm: basic principles, pathophysiology and implications for clinicians. Surgical Infections. 2014; 15(1): 1-7	No concept data
Hampton S. Caring for sloughy wounds. J Community Nurs. 2005; 19(4): 30-34	No concept data
Hermans MH. A retrospective study: rapid removal of biofilm and necrosis with a hygroscopic chemical debriding compound. Journal of Burn Care & Research. 2022; 43: S207.	No concept data
Hernández- Martínez-Esparza E. Santesmases-Masana R. Román E. et al. Prevalence and characteristics of older people with pressure ulcers and leg ulcers in nursing homes in Barcelona. Journal of Tissue Viability. 2021; 30: 108-115	No concept data
Høiby N. Bjarnsholt T. Moser C. et al. ESCMID guideline for the diagnosis and treatment of biofilm infections 2014. Clinical Microbiology and Infection. 2015; 21: S1-S25	No concept data

Honorato-Sampaio K. Martins Guedes CA. de Araújo Nogueira Lima VL. et al. Bacterial biofilm in chronic venous ulcer. The Brazilian Journal of Infectious Diseases. 2014; 18(3): 350-351	No concept data
Hurlow J. Couch K. Laforet K. et al. Clinical biofilms: A challenging frontier in wound care. Advances in Wound Care. 2015; 4(5): 295 - 301	No concept data
Hurlow J. Response to White and Cutting critique. J WOUND CARE. 2012; 21(4): 198	No concept data
Husain FM et al. (2021). MRSA, EBSL, and biofilm formation in diabetic foot ulcer infections. In Zubair M. Ahmat J. Malik A. Talluri MR. (eds) Diabetic foot ulcer. Springer, Singapore.	No concept data
Jones EM. Cochrane CA. & Percival SL. The effect of pH on the extracellular matrix and biofilms. Advances in Wound Care. 2015; 4(7): 431-439	No concept data
Kalkanci A. & Güzel Tunçcan Ö. Biyofilm İlişkili Enfeksiyonlar: Tanı, Tedavi ve Korunma (Biofilm-related infection: diagnosis and treatment). Mediterr J Infect Microb Antimicrob. 2019; 8: 18	No concept data
Karaeng R. Gambaran Perawatan luka pada Tn. AA dengan Ulkus Vena (Venous Ulcer) di klinik perawatan luka ETN Centre Makassar : Laporan Kasus (Overview of wound care in Mr. AA with Venous Ulcer at ETN Center Makassar wound care clinic : Case Report) 2021; Laporan Kasus. Skripsi thesis, universitas Hasanuddin. http://repository.unhas.ac.id:443/id/eprint/2929 (Accessed 5 September 2022)	No concept data
Kim D. William NI. Moore J. et al. Clinical assessment of a biofilm-disrupting agent for the management of chronic wounds compared with standard of care: a therapeutic approach. Wounds: A compendium of clinical research and practice. 2018; 30(5): 120-130	No concept data
Kingsley A. Lewis A. & White R. Debridement and wound biofilms. J WOUND CARE. 2011; 20(6): 286	No concept data
Kirketerp-Møller K. Zulkowski K. & James G. Chronic wound colonization, infection and biofilms. In Bjarnsholt et al. Eds, Biofilm Infections. Springer, 2001	No concept data
Kirketerp-Møller K. & Gottrup F. Bakteriell biofilm i kroniske sår (Bacterial biofilms in chronic wounds). Ugeskr Læger. 2009;17(13): 1097	No concept data
Lantis J. Schultz G. Edmondson-Jones M. et al. Effects of cadexomer iodine on biofilm in diabetic foot ulcers: a pilot study. J WOUND CARE. 2017; 26: 360	No concept data

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Webb R. Hard-to-heal wounds: TIMERS for action. J WOUND CARE. 2019; 28(3): 131	No concept data

Appendix 2: Phase 2

- A draft invitation to participate email p. 323
- A participation information leaflet p. 325
- A draft of the electronic Delphi survey questionnaire p. 329
- A draft of a feedback document provided to participants at the end of round one p. 345
- A draft reminder to complete round one email p. 349
- A draft reminder to complete round two email p. 350
- An approval letter for this study from the University of Galway REC p. 351

Subject box: To gain wound care practitioner consensus on the signs, symptoms and/or biomarkers likely to indicate presence of biofilm in chronic wounds: an electronic Delphi study.



Can we ‘see’ biofilm in chronic wounds?

Dear Colleague,

I am a PhD student at the School of Nursing and Midwifery, University of Galway, Ireland, and my area of interest is diagnosis of biofilm in chronic wounds.

I have completed a comprehensive scoping review of the literature to derive a list of signs and symptoms currently reported to be indicative of biofilm in chronic wounds.

I have generated a list of signs and symptoms based on the results of the scoping review, and I now require your expert opinion to refine it further via a 2-round electronic Delphi survey in which you will be asked to rate each sign/symptom along a 9-point Likert scale ranging from ‘not likely’ to ‘highly likely’ to be indicative of biofilm in chronic wounds.

The results of this survey will be validated against a gold standard biopsy taken from consenting persons with diabetic foot ulceration in the clinical phase of my PhD project.

Should you wish to consider participating, I would direct you to the attached Participant Information Leaflet and if you decide to proceed, I would ask you to click on [this link](#) which will take you to round one of the survey. You will receive a reminder to complete message one week after receiving this invitation, and we will send you a link to round 2 within six weeks followed by a single reminder to complete message.

I sincerely hope that you will agree to participate in this work, and I would ask you to consider forwarding this message to colleagues of yours in the field who may not be members of this organisation.

For any queries you can contact me at J.IVORY2@nuigalway.ie

Appendices

Yours sincerely,

John D. Ivory

Page 1 of 1



Participant information sheet

To gain wound care practitioner consensus on the clinical signs, symptoms and/or biomarkers likely to indicate presence of biofilm in chronic wounds: an electronic Delphi study.

Investigators: Mr. John D. Ivory, Dr. Duygu Sezgin, Ms. Patricia M. Coutts, Dr. Davood Roshan, Ms. Chloe M. Hobbs, Prof. José Verdú Soriano, Prof. James P. O’Gara, Mr. David Gallagher, Prof. Georgina Gethin.

Introduction

There are clinical signs and symptoms reported in the literature as being indicative of biofilm in chronic wounds, but none have been scientifically proven, and clinicians are currently advised to assume that all non-progressing chronic wounds have biofilm present. We suspect however that this assumption may result in overuse of antimicrobial agents without necessarily improving patient outcomes.

We have carried out a comprehensive scoping review of the literature and derived a list of signs and symptoms thought to be indicators of biofilm presence in chronic wounds.

We would like to invite you to participate in an electronic Delphi process that aims to gain consensus from a group of internationally based wound care clinicians on which of a series of signs and symptoms selected from this list are most likely to be indicators of biofilm in chronic wounds.

We would advocate that you take at least 24 hours to consider whether or not you would like to participate in this study.

Procedures

Participation is expected to require 15 – 20 minutes of your time for each round of the survey. You have the right to decline to offer any information asked by this survey should you so wish.

Round 1:

Following a series of eligibility/consent questions you will be asked to provide demographic information i.e., years of experience, country of practice, specialty (nursing, medicine, podiatry etc.), practice area (general practice, gerontology etc.) and setting.

You will then be presented with a list of 26 items reported in the literature as being indicative of biofilm presence in chronic wounds. You will be asked to rate each item along a 9-point Likert scale (1-3 = not likely to be indicative of biofilm in chronic wounds, 4-6 = somewhat likely to be indicative of biofilm in chronic wounds & 7-9 = very likely to be indicative of biofilm in chronic wounds). You will be asked to provide a brief justification for your score in a text box associated with each item, but this step is optional. This anonymous information will be provided as feedback to all participants between rounds. The duration of this round will be approximately 21 days. You will receive a single reminder to complete email from the gatekeeper of your organisation 1 week from receiving this invitation.

Round 2:

Within 6 weeks of completing round 1 you will receive an email from the study investigators containing a link to round 2 of the survey in which you will be provided with aggregated feedback and a summary of the data analysis from round one, and you will be asked to rate items from that round deemed to have no consensus along the same 9-point Likert scale as outlined above. This round will not have an open question for each item, and you will not be asked to provide feedback regarding your score.

You will receive a single reminder to complete email from the study investigators 1 week after receiving the link.

The duration of this round will be 21 days maximum.

Benefits

There are no direct benefits from participating in this study. However, its findings will contribute toward determining if an appropriate diagnostic tool for determining presence of biofilm in chronic wounds at the bedside can be developed. Such a tool would be of significant, direct benefit to wound care clinicians.

Confidentiality

Confidentiality of information cannot be absolutely guaranteed by the research team and can only be protected within the limits of the law.

Because this is a two-round Delphi study, it will be necessary to record your email address in round 1 for the purpose of sending a link to round 2 of the survey and a single reminder to complete email. All email correspondence will be blind carbon copied. Email addresses will only be used for the purposes described above and will not be shared with any other entities in any location. We do not intend to follow up with you at any time in the future regarding this current project or any future research.

Your survey responses will be kept confidential and identifiable information will not be directly linked to individual responses at any time during or after the study. The [QuestionPro](#) platform upon which the questionnaire and survey data will be held is firewall protected and data will only be accessed by allocated researchers who must provide usernames and passwords. All data will be analysed per group rather than per individual case. Physical documents (if any) will not have associated identifiers and will be stored in a locked cabinet with access strictly restricted to the allocated researchers. Printed documentation will be shredded immediately after use. Apart from web-stored data (see above), all computerised data/information will be stored on a password protected hard drive in a locked office with restricted access.

Risks

There are no risks anticipated from participating in this study. Participants will not meet face-to-face at any stage during the process and will be anonymous to each other and to all members of the research team except the project coordinator. All confidentiality procedures will be strictly adhered to as outlined above.

Voluntary participation

Participation in this research is entirely voluntary. You may withdraw from this study without penalty at any point and you will not be required to complete both rounds of the survey if you do not wish to. Participating in or withdrawing from this study will not impact your relationship with your current organisation/employer. You may request to have your data deleted from the study up until the point of its aggregation with all study data for processing and analysis.

Permission to participate.

This study has received ethical approval from the University of Galway Research Ethics Committee. Queries about the study or procedures can be directed to the email address listed in the contact details below.

We would like to thank you for providing us with your time and support. If you volunteer to participate in this study, clicking on the link provided in the invitation email will take you to a series of yes/no questions that will determine your eligibility and ask if you are willing to consent to participate. If you answer ‘no’ to any of these questions you will receive a message of thanks and will not be able to proceed any further.

You may withdraw any time without penalty or waiving of any legal rights. You may also request to have any data that you input into the study withdrawn up until the point of its aggregation with all study data for processing and analysis without penalty or waiving of any legal rights.

Funding of this research

This study is funded by a scholarship (#GOIPG/2020/535) from the Irish Research Council (IRC), Dublin, Ireland, and the Collaborative Doctoral Awards (CDA) Diabetic Foot Disease: From Prevention to Improved Patient Outcomes (DFD PRIMO) program (CDA-2019-007), University of Galway, Galway, Ireland.

Contact details

Research coordinator: Mr. John D. Ivory, J.IVORY2@universityofgalway.ie

Please feel free to contact Mr. Ivory for any questions you may have about this study.

Signs, symptoms and biomarkers most likely to indicate biofilm presence in chronic wounds: an electronic Delphi survey.

We have collated a list of signs, symptoms and biomarkers reported in the literature to be indicative of biofilm in chronic wounds and we need your help to determine which ones are most likely to be so.

Section 1 of 3. Eligibility and consent (5 questions).

To ensure your eligibility and willingness to participate, we would ask you to choose the option most relevant to you or fill in the text box (where appropriate) for each question below.

A 'no' answer to any question in this section will render you unable to complete the questionnaire.

- | | | |
|---|-----|----|
| 1. I am 18 years of age or older. | Yes | No |
| 2. I have 3 or more years clinical experience. | Yes | No |
| 3. 50% or more of my clinical experience has been spent in a wound care environment. | Yes | No |
| 4. I have read and understood the participation information leaflet (please see invitation email attachment). | Yes | No |
| 5. Do you consent to participate in this electronic Delphi survey? | Yes | No |

Section 2 of 3: Demographics (6 questions)

We would like to know a little bit about the professional background and experience of our participant group for the purposes of this study.

Can you choose the option most relevant to you or fill in the text box (where appropriate) for each question below?

1. Clinical experience (years):

3 – 5	☐
6 - 10	☐
11- 15	☐
16 – 20	☐
21 +	☐
Prefer not to say	☐

2. Wound care experience (years):

1.5 - 3	☐
3 - 5	☐
6 – 10	☐
11 – 15	☐
16 – 20	☐
21 +	☐
Prefer not to say	☐

3. Specialty:

Nursing	☐
Podiatry	☐
Medicine	☐
Other	☐
If other, please specify	
Prefer not to say	☐

4. Field of practice:

General practice	☐
Wound, ostomy, continence	☐

	Gerontology	☐
	Dermatology	☐
	Other	☐
	If other, please specify	
	Prefer not to say	☐
5. Practice setting:	Private practice	☐
	Community clinic	☐
	Nursing home	☐
	Hospital (inpatient)	☐
	Hospital (ambulatory)	☐
	Other	☐
	If other, please specify	
	Prefer not to say	☐
6. Country of practice (please enter 'n/a' if you prefer not to answer.)		

Section 3 of 3: Signs and symptoms reported in the literature as being indicative of biofilm in chronic wounds (26 questions)

Thanks for completing sections 1 and 2.

Now, moving forwards, we are going to ask you to rate items in the list that will follow by choosing a score for each that you think to be most appropriate:

1 - 3 = Not likely to be indicative of biofilm in chronic wounds.

4 – 6 = Somewhat likely to be indicative of biofilm in chronic wounds.

7 – 9 = Very likely to be indicative of biofilm in chronic wounds.

In situ indicators (this segment has 16 questions).

1. Debris or foreign objects in the wound (e.g. pins, mesh, prosthetic devices).

Not likely to be indicative.		Very likely to be indicative.
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1	2	3	4	5	6	7	8	9

Would you like to provide a brief justification for your answer to this question (optional)?

2. Low level/increased erythema.

Not likely to be indicative.		Very likely to be indicative.
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1	2	3	4	5	6	7	8	9

Would you like to provide a brief justification for your answer to this question (optional)?

3. Increased exudate.

Not likely to be indicative.		Very likely to be indicative.
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1	2	3	4	5	6	7	8	9

Would you like to provide a brief justification for your answer to this question (optional)?

4. Poor quality granulation tissue (e.g. discoloured, friable, fragile or hypergranulation).

Not likely to be indicative.		Very likely to be indicative.
---	--	--

1	2	3	4	5	6	7	8	9

Would you like to provide a brief justification for your answer to this question (optional)?

5. Signs of local infection (e.g. erythema, oedema, serous effusion, new areas of breakdown, pain etc.).

Not likely to be indicative.		Very likely to be indicative.
---	--	--

1	2	3	4	5	6	7	8	9

Would you like to provide a brief justification for your answer to this question (optional)?

6. Persistent or prolonged (low-level) inflammation.

Not likely to be indicative.		Very likely to be indicative.
---	--	--

1	2	3	4	5	6	7	8	9

Would you like to provide a brief justification for your answer to this question (optional)?

7. Signs of necrotic tissue/eschar.

Not likely to be indicative.		Very likely to be indicative.
---------------------------------	--	----------------------------------

1	2	3	4	5	6	7	8	9

Would you like to provide a brief justification for your answer to this question (optional)?

8. Malodour.

Not likely to be indicative.		Very likely to be indicative.
---------------------------------	--	----------------------------------

1	2	3	4	5	6	7	8	9

Would you like to provide a brief justification for your answer to this question (optional)?

9. Swelling/wound bed with pale oedema.

Not likely to be indicative.

Very likely to be indicative.

1	2	3	4	5	6	7	8	9

Would you like to provide a brief justification for your answer to this question (optional)?

10. Wound pain/pain at site.

Not likely to be indicative.

Very likely to be indicative.

1	2	3	4	5	6	7	8	9

Would you like to provide a brief justification for your answer to this question (optional)?

11. Tunnelling (along tissue structures, degradation along solid structures such as tendon or bone) and/or undermining (erosion of the subcutaneous layer under the wound edge).

Not likely to be indicative.		Very likely to be indicative.
---------------------------------	--	----------------------------------

1	2	3	4	5	6	7	8	9

Would you like to provide a brief justification for your answer to this question (optional)?

12. Sepsis.

Not likely to be indicative.		Very likely to be indicative.
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1	2	3	4	5	6	7	8	9

Would you like to provide a brief justification for your answer to this question (optional)?

13. Presence of slough or fibrin/fibrin coating.

Not likely to be indicative.		Very likely to be indicative.
---	--	--

1	2	3	4	5	6	7	8	9

Would you like to provide a brief justification for your answer to this question (optional)?

14. A shiny, slimy layer on a non-healing wound surface that persists despite autolytic or enzymatic debridement but can be removed easily with physical removal techniques such as cleansing or mechanical debridement. The surface reforms quickly (1-2 days) in the absence of frequent intervention (e.g. cleansing, debridement).

Not likely to be indicative.		Very likely to be indicative.
---	--	--

1	2	3	4	5	6	7	8	9

Would you like to provide a brief justification for your answer to this question (optional)?

15. Temperature, sometimes low-grade fever.

Not likely to be indicative.		Very likely to be indicative.
---	--	--

1	2	3	4	5	6	7	8	9

Would you like to provide a brief justification for your answer to this question (optional)?

16. Wound edge (e.g. maceration, non-physiological colour changes of the wound margin, hyperkeratosis).

Not likely to be indicative.		Very likely to be indicative.
---	--	--

1	2	3	4	5	6	7	8	9

Would you like to provide a brief justification for your answer to this question (optional)?

Patient/wound history indicators

(Well done! Over halfway there, this segment has 10 questions).

17. Negative cultures despite signs/suspicion of clinical infection.

Not likely to be indicative.		Very likely to be indicative.
---	--	--

1	2	3	4	5	6	7	8	9

Would you like to provide a brief justification for your answer to this question (optional)?

18. Wound duration of more than 6 weeks.

Not likely to be indicative.		Very likely to be indicative.
---	--	--

1	2	3	4	5	6	7	8	9

Would you like to provide a brief justification for your answer to this question (optional)?

19. Favourable response to therapies such as corticosteroids, TNF α inhibitors or multimodal strategies such as physical debridement, cleansing and topical agents and dressings.

Not likely to be indicative.		Very likely to be indicative.
---	--	--

1	2	3	4	5	6	7	8	9

Would you like to provide a brief justification for your answer to this question (optional)?

20. Failure to respond as expected to antimicrobial therapies (e.g. systemic antibiotics and/or topical antimicrobials/antiseptics). Infection may re-emerge when treatment is completed.

Not likely to be indicative.		Very likely to be indicative.
---	--	--

1	2	3	4	5	6	7	8	9

Would you like to provide a brief justification for your answer to this question (optional)?

21. History of systemic antibiotics in the last 12 months, prior or current use of antimicrobials.

Not likely to be indicative.		Very likely to be indicative.
---	--	--

1	2	3	4	5	6	7	8	9

Would you like to provide a brief justification for your answer to this question (optional)?

22. Wound fails to close or progress to healing despite optimal wound/patient management.

Not likely to be indicative.		Very likely to be indicative.
---	--	--

1	2	3	4	5	6	7	8	9

Would you like to provide a brief justification for your answer to this question (optional)?

23. Extreme tolerance to host immune defence.

Not likely to be indicative.		Very likely to be indicative.
---	--	--

1	2	3	4	5	6	7	8	9

Would you like to provide a brief justification for your answer to this question (optional)?

24. Infection lasting more than 30 days or persistent cycles of recurrence (waxing and waning).

Not likely to be indicative.		Very likely to be indicative.
---	--	--

1	2	3	4	5	6	7	8	9

Would you like to provide a brief justification for your answer to this question (optional)?

25. Soft tissue deterioration despite antibiotic treatment and/or surgical (*sic*) debridement.

Not likely to be indicative.		Very likely to be indicative.
---------------------------------	--	----------------------------------

1	2	3	4	5	6	7	8	9

Would you like to provide a brief justification for your answer to this question (optional)?

26. Other patient history indicators (change in the patient's overall wellbeing or quality of life).

Not likely to be indicative.		Very likely to be indicative.
---------------------------------	--	----------------------------------

1	2	3	4	5	6	7	8	9

Would you like to provide a brief justification for your answer to this question (optional)?

Before you go, we would ask if you would be willing to provide us with a contact email address. This consensus process has two rounds, and we will need to send you a link to the second round. We will also need to send you a single reminder email to complete the second round. Your email address will only be used for the purposes of this study as outlined above. It will not be used in the data analysis, nor will it be used in any way outside or beyond this study.

Debris or foreign objects in the wound (e.g. pins, mesh, prosthetic devices).

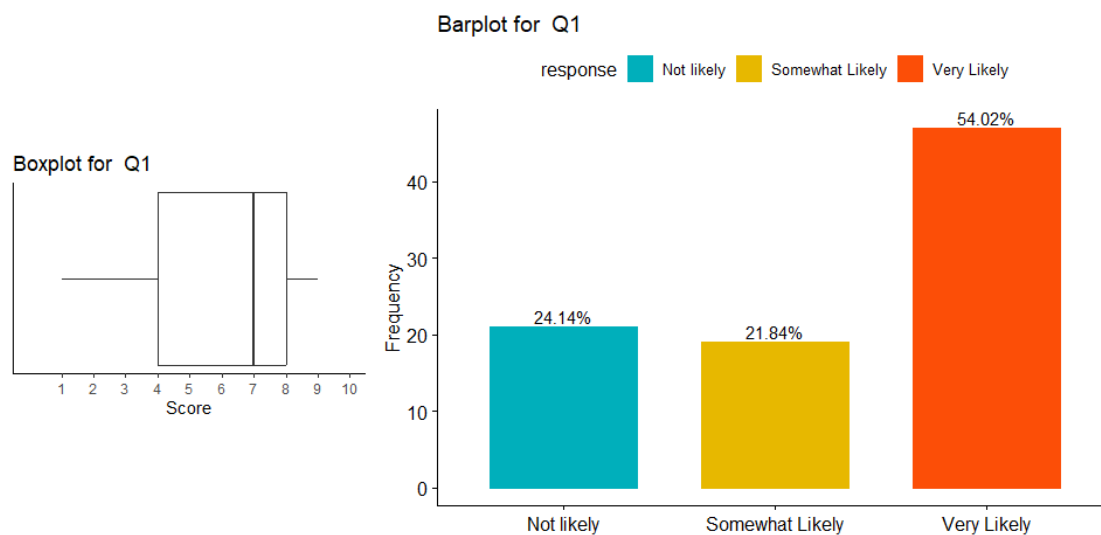
Number of respondents: n = 87 (100%)

Missing: n = 0 (0%)

Rating	1-3 (not likely to be indicative)	4-6 (Somewhat likely to be indicative)	7 -9 (Very likely to be indicative)	Overall
Frequency	21	19	47	87
%	24.1	21.8	54.0	100

Mean (SD) score = 5.86 (2.62)

Median (IQR; Q1, Q3) score = 7.00 (4.00; 4.00, 8.00)



Participant comments

1 - 3 Not likely to be indicative of biofilm

- “Can promote biofilms but are not in themselves.”
- “Un cuerpo extraño provocaría una hipergranulación en el tejido o una infección (A foreign body wound cause hypergranulation in the tissue or an infection).”
- “Debris, pins etc can be found in acute/new wounds not specifically chronic wounds.”
- “Just having a foreign object does not indicate biofilm.”
- “Any strange object in a wound isn’t indicative of biofilm, we can find it in an acute wound.”
- “Si hay cuerpos extraños es que la herida es reciente es mas difícil encontrar biofilm en heridas que no llevan tiempo en exposición (If there are foreign bodies, it is because the wound is recent, it is more difficult to find biofilm in wounds that have not been exposed for a long time.).”
- “Debris etc. do not have a biofilm on the wound.”
- “Detritus or foreign objects do not have to be associated with biofilm if they are handled properly.”
- “External debris can be cleaned.”
- “Wounds with surgical materials can be clean.”

4 – 6 Somewhat likely to be indicative of biofilm

- “Increased risk of bacterial colonisation and favourable conditions where devices etc. are resident in the wound bed.”
- “Depending on the time spent at the injury bed.”
- “Not ruling out biofilm but also foreign objects don’t guarantee biofilm present.”
- “Increased potential for infection with the presence of a foreign body.”
- “Don’t generally work with wounds that are so fresh they have debris.”
- “Cualquier objeto puede desarrollar biofilm, prótesis, lentes intraoculares, lentillas corneales, etc (Any object can develop biofilm, prostheses, intraocular lenses, corneal contact lenses etc.).”
- “It depends how long the FB is in the wound, if acute there may be no biofilm.”
- “Biofilm is known to exist on foreign objects in open wound. This ‘5’ is because the growth of biofilm is dependent of whether this is an acute or chronic wound.”

- “A foreign object/implant may cause delayed healing due to other reasons and not only the likelihood of biofilm.”

7- 9 Very likely to be indicative of biofilm

- “Foreign bodies including devices predispose to infection which may be by biofilm.”
- “Likely to harbour microbes more readily with exposed implants. chronicity could be due to biofilm or the device, but I would suspect the former certainly.”
- “Biofilms readily attach to foreign bodies.”
- “Exposed objects such as pins or mesh common on chronic nonhealing wounds and tend to be ideal for biofilm.”
- “Hard surface for attachment of biofilm.”
- “Biofilm attached to pine etc.”
- “The presence of a foreign body is likely to attract bacteria.”
- “Biofilm can establish itself on non-living objects.”
- “Son zonas de fijación de patógenos (They are pathogen fixation zones).”
- “Any osteosynthesis material, surgical mesh, etc. exposed in a wound is still a foreign body with a risk of infection. Depending on the patient's pathologies and if he or she has toxic habits, there will be a greater chance of this happening.”
- “Despite the neutral material in prosthetics and suturing the body often sets up an inflammatory reaction to these foreign bodies with the resulting infiltration of markers causing a moist, vascular environment which can enhance microbial proliferation despite those markers being part of the complimentary immune response. Debris is a no-brainer unless removed from wound immediately in the acute setting.”
- “Wound breakdown has occurred to see metal, mesh etc. so in the presence of this wound indicates wound breakdown and therefore probable biofilm.”
- “These devices are very likely to have biofilm when exposed. Often, they are exposed after infection.”
- “Zone non pulite dove si accumulano batteri (Unclean areas where bacteria accumulate).”
- “FB is known biofilm attachment.”
- “We know that any time we put a foreign object in the body that biofilm can attach and/or clog.”

- “Foreign objects, including stainless steel have potential for bacterial/biofilm growth, and are more difficult to clean/debride.”

Subject:

Clinical Indicators of Biofilm in Wounds eDelphi study: Reminder to complete. **Please ignore if you are not already a consenting participant.**



Dear Participant,

Please accept our apologies in advance if you have received this email from another source or if you have already completed round 1. Thank you for your time and please ignore this email reminder.

Thank you for agreeing to participate in this project entitled: “To gain wound care practitioner consensus on the clinical signs, symptoms and/or biomarkers likely to indicate presence of biofilm in chronic wounds: an electronic Delphi study.”

This is a reminder message, asking you to complete round 1 of the survey at your earliest convenience.

Sincerely yours,

John D. Ivory

Subject:

Clinical Indicators of Biofilm in Wounds eDelphi study: Reminder to complete.



OLLSCOIL NA GAILLIMHE
UNIVERSITY OF GALWAY



IRISH RESEARCH COUNCIL
An Chomhairle um Thaighde in Éirinn



Dear participant,

Please accept our apologies in advance if you have already completed round 2. Thank you for your time and please ignore this email reminder.

Thank you for agreeing to participate in this research project entitled: “To gain wound care practitioner consensus on the clinical signs, symptoms and/or biomarkers likely to indicate presence of biofilm in chronic wounds: an electronic Delphi study.”

This is a reminder message, asking you to complete round 2 of the survey at your earliest convenience.

Sincerely yours,

John D. Ivory



O'LLSCOIL NA GAILLIMHE
UNIVERSITY OF GALWAY

RESEARCH ETHICS COMMITTEE DECISION LETTER

REC Application Reference Number: 2023.08.015

Title: To gain wound care practitioner consensus on the clinical signs, symptoms and/or biomarkers likely to indicate presence of biofilm in chronic wounds: an electronic Delphi study.

Principal Applicant: John Ivory
School: Nursing and Midwifery

Application Type: NEW
Meeting Date: 28 August 2023

Decision: Full Approval

12 September 2023

Dear Mr Ivory,

I write to you regarding the above proposal which was submitted for ethical review. I'm pleased to advise you that the Committee granted the project **Full Approval**.

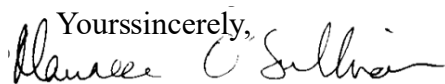
Please note the following regarding REC approval:

1. This submission has been reviewed primarily from an ethical perspective. It is the responsibility of the Principal Applicant to ensure and monitor compliance with any relevant legislation/public health guidelines in the country where the study is due to take place or any local policy in the site where the study is due to take place.
2. It is the researcher's responsibility to be fully compliant with GDPR requirements, including engaging with the DPO / DPIA process.
3. Any significant alterations to an approved proposal must receive approval from the REC prior to implementation. Please request an Amendment Form.
4. You are responsible for notifying the REC in the event of serious or unexpected adverse effects, unforeseen circumstances, the termination of the study, and any significant decisions by other Ethics Committees. Section 7 of the REC's Standard Operating Procedures gives further details on instances requiring follow-up reviews, and reporting obligations.

5. Principal Applicants given University of Galway REC approval must, upon completion of the approved research, submit an End-of Study report. Failure to submit such a report may impact upon future ethics applications.

When the decision was made, I was chairing the meeting, and the following members were also present:

Dr Adrian Clear	Dr Peter Corcoran	Dr Sinead Duane	Dr Ciara Egan
Dr Mark Elliott	Dr Jenny Groarke	Dr Christina Lydon	Dr Verna McKenna (observer)
Dr Anne Mullen	Dr Chris Noone	Dr Judith O'Connell	Dr Maureen O'Sullivan
Dr Saif Rahman	Dr Duygu Sezgin	Dr Amir Shafat	Dr Ihsan Ullah
Dr Jane Walsh			

Yours sincerely,


Dr Maureen O'Sullivan, REC Chair

Appendix 3: Phase 3

- A draft Patient participation information leaflet p. 354
- A draft Patient informed consent form p. 362
- A draft Clinician participant information leaflet p. 367
- A draft Clinician informed consent form p. 374
- Draft Data collection forms p. 378
- A draft letter to primary care providers p. 386
- A study master list for pseudonymising data p. 387
- An indemnity letter from the University of Galway
Insurance Services Office p. 388
- An approval letter for the study protocol from Galway
Clinical Research Ethics Committee (GCREC) p. 391
- An approval letter to amend the study protocol from Galway
Clinical Research Ethics Committee (GCREC) p. 394

Participant information sheet (Patient)

A clinical study to validate signs and symptom reported in the literature to be indicative of biofilm in chronic wounds against a gold standard biopsy in a sample of patients with diabetic foot ulceration.

Investigators: Professor Sean Dineen, Dr. Niamh Phelan, Dr. Pauline Wilson, Professor James P. O’Gara, Professor Georgina Gethin, Professor Caroline McIntosh, Dr. Michael John Lockhart, Dr. Merve Zeden, Mr. John D. Ivory.

Invitation

We would like to invite you to participate in this study which will be carried out at the Diabetes Day Centre, St. James’s Hospital (SJH), Dublin.

- Before you decide whether or not you would like to take part in this study, it is important that you understand why this research is being conducted.
- We would encourage you to take your time to read the following information and if you have any questions, you can contact Dr. Phelan or Dr. Wilson at the St. James’s clinic, Professor Dineen at The University of Galway, or Mr. John Ivory at the University of Galway using the contact details listed below.
- Participation in this study is completely voluntary. If you decide not to participate it, will not affect the care you receive at St. James’s Hospital, Dublin or your rights, safety and wellbeing as a patient.

What are the goals of this research study?

When we think of bacteria or germs, we usually think of single cells living separately from each other in an environment such as water, on surfaces that we use, or in a wound. However, bacteria also exist in complex communities called biofilms which are surrounded by a protective covering and the cells within live cooperatively with each other. These two factors help bacteria to survive, they can make biofilm infections difficult to treat, and it is generally accepted that when biofilms become established in chronic wounds such as a diabetic foot ulcers, they can add further delays to their healing.

They can be found in 60 to 78% of chronic wounds (maybe even in as many as 90%) but are difficult to diagnose on-the-spot in the clinic. At the moment, the best way to find them

involves complicated laboratory procedures, and the swab of your wound that we take can only find single bacterial cells, not the communities (biofilms). This means that doctors and nurses often rely on clinical indicators (what they can see in your wound or medical history for example swelling, redness or if your wound isn't responding well to antibiotics) to help them decide if there is biofilm in your wound.

We have put a list of 11 clinical indicators together that wound care clinicians currently think can point to biofilm in chronic wounds and we want to test them against a gold-standard laboratory procedure to see which of them really can. We also want to see if different clinicians will spot the same signs from the list when they look for them in your wound.

Who is conducting this study?

This study will be conducted by Mr. John Ivory, a student at the School of Nursing & Midwifery, University of Galway as part of his PhD degree. The study is funded by a scholarship (#GOIPG/2020/535) from the Irish Research Council (IRC), Dublin, Ireland, and the Collaborative Doctoral Awards (CDA) Diabetic Foot Disease: From Prevention to Improved Patient Outcomes (DFD PRIMO) program (CDA-2019-007), University of Galway.

Who can take part?

You can take part if you are over 18 years of age, have type 2 diabetes, if you currently have an open foot ulcer, and if in the opinion of your attending clinician or the investigators of this study, it is safe for you to participate.

What steps will be taken as part of this study?

If you agree to participate in this study and sign a consent form, we will ask you for your age, sex and duration of your diabetes while your dressings are being removed. We will also gather details about your wound including its location, how long it has been there, its size and how the nurse, podiatrist or doctor classifies it according to an assessment tool called the "University of Texas Staging System for Diabetic Foot Ulcers". We will also assess your wound with a checklist of indicators that we think can indicate biofilm presence in your wound.

Two other clinical personnel from the diabetes care centre will separately assess your wound with the same checklist of indicators.

Following the assessment, a clinician will take a tissue sample or biopsy from the edge of your wound. They will first give you a local anaesthetic injection near the biopsy site (so that you won't feel any pain during the procedure and to help any bleeding to stop), and they will cleanse your wound with saline solution. They will use a device called a punch biopsy to take the tissue sample. The clinician will push the device (which measures 4mm in diameter) into the skin to a depth of up to 5mm rotating it 180° both clockwise and anticlockwise while doing so. The clinician will pull the tissue up with the help of a forceps or needle and they will use scissors to cut the base. After taking the biopsy, the clinician will stop any bleeding or oozing by putting pressure on the site using pieces of gauze and an extra pressure layer of gauze and tape will be applied over your usual dressing which must be left in place for 48 hours.

The clinician will mark the skin adjacent to the where the biopsy was taken with a sterile surgical skin marker to highlight the site, and for safety purposes they may take a photograph of the wound which will be kept in your medical chart.

The study will then be complete.

Will I have to do anything to take part in this study?

If you decide that you would like to take part in this study, we will only carry out the procedures outlined in the previous section. If you decide during the study visit that you do not want to answer any of the questions or have any of the procedures done, you can say so and we will not proceed. This will not affect your medical care or rights as a patient at St. James's Hospital in any way.

Are there any risks associated with participation in this study?

Taking a tissue sample by means of a biopsy is invasive and does come with risks because of the nature of diabetes. You may have nerve damage and problems with blood circulation in your feet which means that wounds can develop into ulcers that take a long time to heal. However, there are other published studies which received approval from ethics boards that have used similar biopsy methods to this study and most importantly, if a doctor, nurse or podiatrist raises any concerns about your ability to heal wounds in the lower limbs, you will

not be permitted to enter the study. In addition, you will be closely monitored at subsequent clinic visits, and we will give you a letter to inform community care nurses and your GP that you have had a biopsy taken from your wound.

Local anaesthesia will be administered with a hypodermic syringe, and you may experience some pain or discomfort as a result.

All invasive procedures come with a risk of infection. We will take all standard steps during your visit to prevent an infection from developing in your wound.

The study visit is estimated to take approximately 30 minutes, and this may cause an interruption to your usual schedule on the day.

Are there any benefits associated with participation in this study?

There are no direct benefits to you for taking part in this study. However, the findings of this study may be of benefit to the community in the future.

What if I do not want to participate in this study?

You are free to decline to take part in this study, and you do not have to explain why. You are also free to start the study and withdraw from it at any time without having to give a reason.

A decision to decline to participate or to withdraw after the study has started will not affect your care or your rights as a patient at St. James's Hospital at any time.

Protection of your personal data

It is important that you understand how your information will be used during this study. If you have any questions, please do not hesitate to contact the researchers using the contact details at the end of this document.

- 1. What information will we request and why are we asking for this information?** We will ask for your age, your sex, and the duration of your diabetes. We will also seek information about your wound - its location, how long it has been there, its size and how the attending clinician classified it according to the University of Texas University of Texas Staging System for Diabetic Foot Ulcers. We will process this information statistically to

create an overall picture (for example, average age, average wound size) for other researchers of the participant population for this study.

We will take the biopsy tissue to Microbiology in the School of Natural Sciences, University of Galway where it will be stored and prepared for laboratory analysis. The laboratory analysis will determine if the biopsy tissue you provided contains biofilms or not.

The assessments made of your wound by clinical personnel on the day of your study visit will then be compared with the laboratory findings and this will help us determine which of the signs and symptoms from the list are statistically most likely to point to biofilm presence in chronic wounds.

We may take a photograph of your wound to document where exactly the biopsy was taken from for safety purposes. This photograph will be kept on your medical chart and will not be otherwise processed or used as part of this study.

2. What do I need to know about my confidentiality?

- None of the information we collect will have your name or personal identifiers associated with it. We will assign you a study number if you agree to participate, and this will be used on all samples and data collected during the study.
- We will keep a master list linking your name and date of birth to the study number we assign you. This list will be kept securely and separately from your study data.
- We will have to transfer your biopsy sample from St. James's hospital to the University of Galway for processing and analysis. We will keep your study data on the University of Galway's password-protected cloud server 'OneDrive'.
- We will not store any information about you or your clinical care that is identifiable on this server or in our offices or laboratories at the University of Galway.
- We will not include information about you or your clinical care that is identifiable in any research studies that we publish as a result of this project.

3. The lawful basis for processing data for this study is as follows as defined by the General Data Protection Regulations 2016:

- We believe that this data is important for scientific research (*GDPR Article 9(2)(j)*).
- Processing for reliability and safety purposes (*GDPR Article 6(1)(c) in conjunction with GDPR Article 9(2)(i)*).
- Processing operations purely related to research activities (*GDPR Article 6(1)e in conjunction with GDPR article 9(2)(i) and/or GDPR Article 9(2)(j) as applicable where there is reliance upon derogation for specific situations GDPR Article 49*).

- Underpinning legislation (*GDPR 6(1)(e), GDPR Article 9(2)(i), GDPR Article 9(2)(j). Lawful bases are underpinned by the Universities Act 1997*).
- Explicit consent (Explicit consent is processed in accordance with the Health Research Regulations 2018 Section 3(1)(e) – explicit consent has been obtained from the data subject prior to the commencement of the health research for the processing of his or her personal data for the purpose of specified health research either in relation to a particular area or more generally in that area or a related area of health research, or part thereof.
- Explicit consent is also obtained in accordance with the requirements of Medical Device Regulation Article 63 and S.I. No. 261 of 2021 Medical Devices Regulations 2021.

4. Who will have access to the data?

Information collected for the purposes of this study will be available only to your doctor, nurse or podiatrist, and with your permission to the study investigators.

5. Who is the data controller for this project?

The University of Galway and St. James's Hospital are the joint data controllers for this project.

The St. James's Hospital Data Protection Office is located at:

Ground Floor, CEO Building,

St. James's Hospital,

James St.,

Dublin 8

D08 NHY1

and can be contacted by telephone: (01) 410 3021 or email: dataprotection@stjames.ie

The University of Galway Data Protection Office is located at:

Room A129,

The Quadrangle,

University of Galway,

University Rd., Galway, H91 TK33

and can be contacted by telephone: (091) 524411 or
email: dataprotection@universityofgalway.ie

6. How long will the data be stored for?

With your permission, the study master list, signed consent forms and data linked to biopsies from your wound (your age, gender, duration of your diabetes, wound location, duration, size and how the attending clinician classified it according to the University of Texas University of Texas Staging System for Diabetic Foot Ulcers will be stored securely at The Diabetes Day Centre, St. James's Hospital (for patients attending this hospital) for a period of 7 years after which it will be destroyed.

The tissue biopsies collected in this study will have no personal identifiers associated with them and they will be stored in a freezer at Microbiology, School of Natural Sciences, University of Galway until the study is completed. At this point they will be destroyed. We will not be using these samples in any future studies.

Study data stored on the University of Galway OneDrive password-protected cloud server which will have no personal identifiers associated with it will be stored securely for a period of 7 years.

7. What are my rights?

- a) You have the right to withdraw from this study at any time by telling the researchers or attending clinician before or during the study visit.
- b) You have a right to lodge a complaint with the Data Protection Commissioner if you are dissatisfied.
 - The Data Protection Commission (DPC) can be contacted as follows:
 - Telephone:
+353 57 8684800
+353 (0) 761 1 04 800 (hours 0915 -1730 [1715 Friday]) Lo
Call Number: 1890 252 231
 - Fax: +353 57 868 4757
 - Website: <https://www.dataprotection.ie/en/contact/how-contact-us>
- c) You have the right to have any inaccurate information about you corrected or deleted.
- d) You have the right to have any information about you deleted if you no longer wish to participate in this study or no longer consent to the data being stored for the purpose of this study.
- e) You have the right to have your data moved from one controller to another in a readable format.

- f) You have the right to request any of your information in a readable format.
- g) You have the right to object to restrict processing of your data.

8. How will you use my information?

- a) There will be no automated decision-making or profiling of your personal information in relation to this study. This means that we will not use your personal data to make predictions about you.
- b) Other than for this study, your information will not be processed for any other reason.
- c) Your personal information will not be transferred to any other organisation or country.
- d) Your information will be held on a password -protected file storage system. Only the data controllers for this study will have access to this.

9. Who can I contact with questions or if I wish to withdraw from the study?

Dr. Niamh Phelan, phelansec@stjames.ie, phone: (01) 4162488

Dr. Pauline Wilson, pwilson@stjames.ie, phone: (01) 4162465

Professor Sean Dineen, sean.dineen@universityofgalway.ie, phone: (091) 544 698

Mr. John D. Ivory, J.IVORY2@universityofgalway.ie, phone: (091) 495481

10. Where can I get more information about participating in an academic research project being undertaken by University of Galway students or staff?

More information about participating in academic research undertaken by students or staff at the University of Galway can be found at the following website:

<https://www.universityofgalway.ie/media/oifiganrunai/NUI-Galway-Research-Participant-PrivacyNotice.pdf>

Participant Consent Form (Patient)

Study title: A clinical study to validate signs and symptom reported in the literature to be indicative of biofilm in chronic wounds against a gold standard biopsy in a sample of patients with diabetic foot ulceration.

I have read and understood the Information Leaflet about this research project. The information has been fully explained to me and I have been able to ask questions, all of which have been answered to my satisfaction.	Yes ☐	No ☐
I understand that I don't have to take part in this study and that I can opt out at any time. I understand that I don't have to give a reason for opting out and I understand that opting out won't affect my future medical care.	Yes ☐	No ☐
I am aware of the potential risks, benefits and alternatives of this research study.	Yes ☐	No ☐
I have been assured that information about me will be kept private and confidential.	Yes ☐	No ☐
I have been given a copy of the Information Leaflet and this completed consent form for my records.	Yes ☐	No ☐
I consent to research team members and participating clinicians in this study having access to my healthcare records for the purposes of this study.	Yes ☐	No ☐
I consent to have a biological sample in the form of a punch biopsy taken from my wound as part of this research study.	Yes ☐	No ☐
I consent to have the biological sample in the form of a punch biopsy transferred to the University of Galway for processing and analysis.	Yes ☐	No ☐
I consent to having my hospital consultant informed of my participation in this study.	Yes ☐	No ☐
I consent to having a photograph taken of my wound for safety purposes and to having it stored in my medical chart.	Yes ☐	No ☐
I consent to having my general practitioner informed of my participation in this study.	Yes ☐	No ☐
I consent to take part in this research study having been fully informed of the risks, benefits and alternatives.	Yes ☐	No ☐

I give informed explicit consent to have my data stored and processed at the University of Galway as part of this research study.	Yes ☐	No ☐
I consent to be contacted by researchers as part of this research study	Yes ☐	No ☐
<i>Storage and Future Use of Information (please tick YES to ONE option)</i>		
OPTION 1 - I give permission for my <u>anonymous</u> data to be stored for <u>possible future research related</u> to the current study <u>only if consent is obtained</u> at the time of the future research and the research is approved by a Research Ethics Committee	Yes ☐	No ☐
OPTION 2 - I give permission for my <u>anonymous</u> data to be stored for <u>possible future research related</u> to the current study <u>without further consent</u> being required but only if the research is approved by a Research Ethics Committee.	Yes ☐	No ☐
OPTION 3 – I request that all my data collected for this study can no longer be used by researchers in the future and that it be destroyed	Yes ☐	No ☐

Patient Name (Block Capitals)

Patient Signature

Date

To be completed by the Principal Investigator or nominee.

I, the undersigned, have taken the time to fully explain to the above patient the nature and purpose of this study in a way that they could understand. I have explained the risks involved as well as the possible benefits. I have invited them to ask questions on any aspect of the study that concerned them.

Name (Block Capitals)

Qualifications

Signature

Date

Principal investigator and Data Controller:

Prof. Sean Dineen, Dr. Niamh Phelan, Mr. John Ivory

Principal investigator's title: Professor, Dr.,

Telephone number/email of principal investigator:

Prof. Dineen – 091 544698/sean.dineen@universityofgalway.ie

Dr. Phelan - 01 4162488/ NPhelan@STJAMES.ie

Data Controller's/joint Controller's Identity:

University of Galway, Galway

St. James's Hospital, Dublin

Data Controller's/joint Controller's Contact Details:

Mr. John Ivory,

Rm. # 234, School of Nursing & Midwifery,

Áras Moyola,

University of
Galway,
University Rd.,
Galway H91TK33.

Tel: 091 495481

Email: J.IVORY2@universityofgalway.ie

Dr. Niamh Phelan,

Diabetes Day Centre,

St. James's Hospital,

James St.,

Dublin 8 D08NHY1

Tel: (01) 4162488

Email: NPhelan@STJAMES.ie

Data Protection Officer's Identity:

University of Galway Data Protection Officer

St. James's Hospital Data Protection Officer

Data Protection Officer's Contact Details:

The Data Protection Officer,

Room A129, The Quadrangle,

University of
Galway,
University Rd.,
Galway H91 TK33.

Tel (091524411

Email: dataprotection@universityofgalway.ie

The Data Protection Officer,
Ground Floor, CEO Building,
St. James's Hospital,
James's St.,

Dublin 8, D08 NHY1

Tel (01) 410 3021

Email: dataprotection@stjames.ie

Participant information sheet (Clinician Inter-rater reliability)

A clinical study to validate signs and symptom reported in the literature to be indicative of biofilm in chronic wounds against a gold standard biopsy in a sample of patients with diabetic foot ulceration.

Investigators: Professor Sean Dineen, Dr. Niamh Phelan, Dr. Pauline Wilson, Professor James P. O’Gara, Professor Georgina Gethin, Professor Caroline McIntosh, Dr. Michael John Lockhart, Dr. Merve Zeden, Mr. John D. Ivory.

Invitation

We would like to invite you to participate in this study which will be carried out at the Diabetes Day Centre, St. James’s Hospital (SJH), Dublin.

- Before you decide whether or not you would like to take part in this study, it is important that you understand why this research is being conducted.
- We would encourage you to take your time to read the following information and if you have any questions, you can contact Dr. Phelan or Dr. Wilson at the St. James’s clinic, Professor Dineen at The University Hospital Galway Clinic, or Mr. John Ivory at the University of Galway using the contact details listed below.
- Participation in this study is completely voluntary. If you decide not to participate it, will not affect your rights, safety or wellbeing as an employee at St. James’s Hospital, Dublin.

What are the goals of this research study?

Biofilms are fixed, sessile bacterial communities that surround themselves in a protective exopolymeric matrix and affix themselves to both biotic and abiotic surfaces. They are present in 60 –78% of chronic wounds (possibly 90%) and are thought to contribute to delayed healing.

Currently accepted methods to identify biofilms require advanced microscopy of tissue samples in conjunction with fluorescent probes but these are not readily available to clinicians and standard culturing methods provide data mostly in terms of planktonic bacterial phenotypes.

There are clinical signs and symptoms reported in the literature as being indicative of biofilm in chronic wounds, but very little validation work has been done. Failure to identify wounds containing biofilm may result in ineffective and delayed healing. Conversely, we are concerned

that assuming biofilm to be present when it is not may lead to excessive use of treatments, thereby compromising good antimicrobial stewardship.

We derived a list of 26 signs and symptoms thought to be indicators of biofilm presence in chronic wounds through a scoping review of the literature and an electronic Delphi consensus of internationally based wound care clinicians has refined this list to 11 potential indicators.

This study aims to determine the inter-rater reliability and the validity of a list of 11 signs and symptoms reported in the literature and voted very likely to be indicative of biofilm in chronic wounds by an international panel of wound care clinicians against a gold-standard biopsy.

Who is conducting this study?

This study will be conducted by Mr. John Ivory, a student at the School of Nursing & Midwifery, University of Galway as part of his PhD degree. The study is funded by a scholarship (#GOIPG/2020/535) from the Irish Research Council (IRC), Dublin, Ireland, and the Collaborative Doctoral Awards (CDA) Diabetic Foot Disease: From Prevention to Improved Patient Outcomes (DFD PRIMO) program (CDA-2019-007), University of Galway.

Who can take part?

You can take part of this study if you are employed as clinical personnel at the Diabetes Day Care Centre, St. James's hospital and are experienced in assessing chronic wounds.

What steps will be taken as part of this study?

If you agree to participate in this study, we will record details about your profession and number of years' experience in wound care.

We will then ask you to examine the wound of the patient participant against a list of 11 signs and symptoms thought to be indicative of biofilm in chronic wounds, checking off any signs that you observe. The wound will be rated by two other clinicians using the same checklist, and from these ratings we will estimate the inter-rater reliability of each sign and symptom on the list.

We will ask you to carry out this procedure once for each patient participant (n = 12 at St. James's hospital). Following your assessment, the study visit will continue with the assistance of the attending clinician.

We will provide you with instruction on how to use the checklist and you will have the opportunity to familiarise yourself with it by assessing wounds in up to 5 patients who are not currently involved in the study and are agreeable to having their wounds assessed.

We estimate that a wound assessment with this tool should take approximately 5 minutes to complete.

If you decide that you would like to take part in this study, we will only ask you to carry out the procedures outlined in the previous section. If you decide during the study that you do not want to proceed you can withdraw consent. This will not affect your rights, safety or wellbeing as a clinician employed at the Diabetes Day Centre, St. James's Hospital, Dublin.

Are there any risks associated with participation in this study?

There are no risks anticipated from your participation in this study. We will require approximately 10 minutes of your time to carry out the assessment.

Are there any benefits associated with participation in this study?

There are no direct benefits anticipated from your participation in this study. However, the findings of this research may be of benefit to the community in the future.

What if I do not want to participate in this study?

You are free to decline to take part in this study, and you do not have to explain why. You are also free to start the study and withdraw from it at any time without having to give a reason.

A decision to decline to participate or to withdraw after the study has started will not affect your rights, safety or wellbeing as an employee as a clinician employed at the Diabetes Day Centre, St. James's Hospital, Dublin at any time.

Protection of your personal data

It is important that you understand how your information will be used during this study. If you have any questions, please contact the researchers using the contact details at the end of this document.

1. What information will we request and why are we asking for this information?

We will ask for your profession and number of years' experience in wound care. This will be processed statistically (for example, average years of wound care experience) to characterise the clinical assessor population of this study.

2. What do I need to know about my confidentiality?

- None of the information we collect will have your name or personal identifiers associated with it. You will be assigned a study number if you agree to participate, and this will be used on all samples and data collected during the study.
- We will keep a master list linking your name and date of birth to the study number we assign you. This list will be kept securely and separately from any study-related information that we collect.
- We will keep your study data on the University of Galway's password-protected cloud server 'OneDrive'.
- We will not store any information about you or your clinical care that is identifiable on this server or in our offices or laboratories at the University of Galway.
- We will not include information about you or your clinical care that is identifiable in any research studies that we publish as a result of this project.

3. The lawful basis for processing data for this study is as follows as defined by the General Data Protection Regulations 2016:

- We believe that this data is important for scientific research (*GDPR Article 9(2)(j)*).
- Processing for reliability and safety purposes (*GDPR Article 6(1)(c) in conjunction with GDPR Article 9(2)(i)*).
- Processing operations purely related to research activities (*GDPR Article 6(1)e in conjunction with GDPR article 9(2)(i) and/or GDPR Article 9(2)(j) as applicable where there is reliance upon derogation for specific situations GDPR Article 49*).
- Underpinning legislation (*GDPR 6(1)(e), GDPR Article 9(2)(i), GDPR Article 9(2)(j). Lawful bases are underpinned by the Universities Act 1997*).
- Explicit consent (Explicit consent is processed in accordance with the Health Research Regulations 2018 Section 3(1)(e) – explicit consent has been obtained from the data subject prior to the commencement of the health research for the processing of his or her personal data for the purpose of specified health research

either in relation to a particular area or more generally in that area or a related area of health research, or part thereof.

- Explicit consent is also obtained in accordance with the requirements of Medical Device Regulation Article 63 and S.I. No. 261 of 2021 Medical Devices Regulations 2021.

4. Who will have access to the data?

Information collected for the purposes of this study will be available only to the study investigators.

5. Who is the data controller for this project?

The University of Galway and St. James's Hospital are the joint data controllers for this project.

The St. James's Hospital Data Protection Office is located at:

Ground Floor, CEO Building,

St. James's Hospital,

James St.,

Dublin 8

D08 NHY1

and can be contacted by telephone: (01) 410 3021 or email: dataprotection@stjames.ie

The University of Galway Data Protection Office is located at:

Room A129,

The Quadrangle,

University of Galway,

University Rd., Galway, H91 TK33

and can be contacted by telephone: (091) 524411 or

email: dataprotection@universityofgalway.ie

6. How long will the data be stored for?

With your permission, the study master list, signed consent forms and data linked your assessment of the wound (your profession and number of years' experience in wound care) will be stored securely at The Diabetes Centre, University Hospital Galway (for clinical

personnel participants at this hospital) and The Diabetes Day Centre, St. James's Hospital (for clinical personnel participants at this hospital) for a period of 7 years after which it will be destroyed.

Study data stored on the University of Galway OneDrive password-protected cloud server which will have no personal identifiers associated with it will be stored securely for a period of 7 years.

7. What are my rights?

- a) You have the right to withdraw from this study at any time by telling the researchers before or during the study visit.
- b) You have a right to lodge a complaint with the Data Protection Commissioner if you are dissatisfied.
 - The Data Protection Commission (DPC) can be contacted as follows:
 - Telephone:
+353 57 8684800
+353 (0) 761 1 04 800 (hours 0915 -1730 [1715 Friday]) Lo
Call Number: 1890 252 231
 - Fax: +353 57 868 4757
 - Website: <https://www.dataprotection.ie/en/contact/how-contact-us>
- c) You have the right to have any inaccurate information about you corrected or deleted.
- d) You have the right to have any information about you deleted if you no longer wish to participate in this study or no longer consent to the data being stored for the purpose of this study.
- e) You have the right to have your data moved from one controller to another in a readable format.
- f) You have the right to request any of your information in a readable format.
- g) You have the right to object to restrict processing of your data.

8. How will you use my information?

- h) There will be no automated decision-making or profiling of your personal information in relation to this study. This means that we will not use your personal data to make predictions about you.
- i) Other than for this study, your information will not be processed for any other reason.
- j) Your personal information will not be transferred to any other organisation or country.
- k) Your information will be held on a password -protected file storage system. Only the data controllers for this study will have access to this.

9. Who can I contact with questions or if I wish to withdraw from the study?

Dr. Niamh Phelan, phelansec@stjames.ie, phone: (01) 4162488

Dr. Pauline Wilson, pwilson@stjames.ie, phone: (01) 4162465

Professor Sean Dineen, sean.dineen@universityofgalway.ie, phone: (091) 544 698

Mr. John D. Ivory, J.IVORY2@universityofgalway.ie, phone: (091) 495481

10. Where can I get more information about participating in an academic research project being undertaken by University of Galway students or staff?

More information about participating in academic research undertaken by students or staff at the University of Galway can be found at the following website:

<https://www.universityofgalway.ie/media/oifiganrunai/NUI-Galway-Research-Participant-PrivacyNotice.pdf>

Participant Consent Form (Clinician Inter-rater reliability)

Study title: A clinical study to validate signs and symptom reported in the literature to be indicative of biofilm in chronic wounds against a gold standard biopsy in a sample of patients with diabetic foot ulceration.

I have read and understood the Information Leaflet about this research project. The information has been fully explained to me and I have been able to ask questions, all of which have been answered to my satisfaction.	Yes ☐	No ☐
I understand that I don't have to take part in this study and that I can opt out at any time. I understand that I don't have to give a reason for opting out and I understand that opting out won't affect my future medical care.	Yes ☐	No ☐
I am aware of the potential risks, benefits and alternatives of this research study.	Yes ☐	No ☐
I have been assured that information about me will be kept private and confidential.	Yes ☐	No ☐
I have been given a copy of the Information Leaflet and this completed consent form for my records.	Yes ☐	No ☐
I consent to take part in this research study having been fully informed of the risks, benefits and alternatives.	Yes ☐	No ☐
I give informed explicit consent to have my data processed at the University of Galway as part of this research study.	Yes ☐	No ☐
I consent to be contacted by researchers as part of this research study	Yes ☐	No ☐
<i>Storage and Future Use of Information (please tick YES to ONE option)</i>		
OPTION 1 - I give permission for my <u>anonymous</u> data to be stored for <u>possible future research related</u> to the current study <u>only if consent is obtained</u> at the time of the future research and the research is approved by a Research Ethics Committee	Yes ☐	No ☐
OPTION 2 - I give permission for my <u>anonymous</u> data to be stored for <u>possible future research related</u> to the current study <u>without further consent</u> being required but only if the research is approved by a Research Ethics Committee.	Yes ☐	No ☐
OPTION 3 – I request that all my data collected for this study can no longer be used by researchers in the future and that it be destroyed	Yes ☐	No ☐

Appendices

Clinician Name (Block Capitals)

Clinician Signature

Date

To be completed by the Principal Investigator or nominee.

I, the undersigned, have taken the time to fully explain to the above patient the nature and purpose of this study in a way that they could understand. I have explained the risks involved as well as the possible benefits. I have invited them to ask questions on any aspect of the study that concerned them.

Name (Block Capitals)

Qualifications

Signature

Date

Principal investigator and Data Controller:

Prof. Sean Dineen, Dr. Niamh Phelan, Mr. John Ivory

Principal investigator's title: Professor, Dr.,

Telephone number/email of principal investigator:

Prof. Dineen – 091

544698/sean.dineen@universityofgalway.ie Dr.

Phelan - 01 4162488/ NPhelan@STJAMES.ie

Data Controller's/joint Controller's Identity:

University of Galway, Galway

St. James's Hospital, Dublin

Data Controller's/joint Controller's Contact Details:

Mr. John Ivory,

Rm. # 234, School of Nursing & Midwifery,

Áras Moyola,

University of

Galway,

University Rd.,

Galway H91TK33.

Tel: 091 495481

Email: J.IVORY2@universityofgalway.ie

Dr. Niamh Phelan,

Diabetes Day Centre,

St. James's Hospital,

James St.,

Dublin 8 D08NHY1

Tel: (01) 4162488
Email: NPhelan@STJAMES.ie

Data Protection Officer's Identity:

University of Galway Data Protection Officer
St. James's Hospital Data Protection Officer

Data Protection Officer's Contact Details:

The Data Protection Officer,
Room A129, The Quadrangle,
University of
Galway,
University Rd.,
Galway H91 TK33.
Tel (091524411
Email: dataprotection@universityofgalway.ie

The Data Protection Officer,
Ground Floor, CEO Building,
St. James's Hospital,
James's St.,
Dublin 8, D08 NHY1
Tel (01) 410 3021
Email: dataprotection@stjames.ie

Data collection form 1 (Validation/Inter-rater + biopsy)

Date	
Patient participant study id #	
Clinician study id #	

Eligibility criteria and consent

Is the patient ≥ 18 years of age? (Y/N)	
Has the patient been diagnosed with type 2 diabetes? (Y/N)	
Does the patient have active diabetic foot ulceration defined by the International Working Group on the Diabetic Foot (IWGDF) as “Infection, ulceration or destruction of tissues of the foot of a person with currently or previously diagnosed diabetes mellitus, usually accompanied by neuropathy and or peripheral arterial disease (PAD) in the lower extremity? (Y/N)	
Does the patient have a University of Texas Staging System for diabetic foot ulcers score of Grade I, Stage B or less? (Y/N)	
Does the patient have blood dyscrasias, venous congestion or extremely vascular wounds in a setting where there is limited access to care or high risk that the biopsy site will not heal? (Y/N)	

Does the patient have absolute contraindications to epinephrine i.e. untreated hyperthyroidism, and pheochromocytoma? (Y/N)	
Is the patient eligible for inclusion into this study?	
Has the patient participant provided signed and dated informed consent? (Y/N)	

Is the clinician employed as clinical personnel at the Diabetes Centre, University Hospital Galway, or at the Diabetes Day Centre, St. James's Hospital Dublin? (Y/N)	
Is the clinician experienced in wound care assessment? (Y/N)	
Is the clinician eligible for participation in this study?	
Has the clinician provided signed and dated informed consent? (Y/N)	

Clinician participant demographics (for inter – rater reliability purposes)

Clinician participant profession	
# years wound care experience	

Patient participant demographics

Age	
Sex	
Duration of diabetes	
Wound location	
Wound duration	
Wound dimensions (longest length x greatest width [cm])	
University of Texas staging system score	Grade: Stage:

Biofilm signs and symptoms checklist

Clinical signs and symptoms of biofilm in chronic wounds checklist	Please check box ✓ if present
Wound duration of more than 6 weeks.	

Wound fails to close or progress to healing despite optimal wound/patient management.	
Infection lasting more than 30 days or persistent cycles of recurrence.	
Failure to respond as expected to antimicrobial therapies (e.g. systemic antibiotics and/or topical antimicrobials/antiseptics). Infection may re-emerge when treatment is completed.	
Signs of local infection (e.g. erythema, oedema, serous effusion, new areas of breakdown, pain, etc.).	
Persistent or prolonged (low-level) inflammation.	
Soft tissue deterioration despite antibiotic treatment and/or surgical debridement.	
A shiny, slimy layer on a non-healing wound surface that persists despite autolytic or enzymatic debridement but can be removed easily with physical removal techniques such as cleansing or mechanical debridement. The surface reforms quickly (1-2 days) in the absence of frequent intervention (e.g. cleansing, debridement).	
Presence of slough or fibrin/fibrin coating.	
Poor quality granulation tissue (e.g. discoloured, friable, fragile or hypergranulation).	
Tunnelling (along tissue structures, degradation along solid structures such as tendon or bone) and/or undermining (erosion of the subcutaneous layer under the wound edge).	

Appendices

Comments (if any): _____

Biopsy taken? (Y/N)	
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Data collection form 2 (Inter-rater reliability only)

Date	
Patient participant study id #	
Clinician study id #	

Eligibility criteria and consent

Is the clinician employed as clinical personnel at the Diabetes Centre, University Hospital Galway, or at the Diabetes Day Centre, St. James's Hospital Dublin? (Y/N)	
Is the clinician experienced in wound care assessment? (Y/N)	
Is the clinician eligible for participation in this study?	
Has the clinical assessor provided signed and dated informed consent? (Y/N)	

Clinician participant demographics

Clinician profession	
----------------------	--

# years wound care experience	
-------------------------------	--

Biofilm signs and symptoms checklist

Clinical signs and symptoms of biofilm in chronic wounds checklist	Please check box ✓ if present
Wound duration of more than 6 weeks.	
Wound fails to close or progress to healing despite optimal wound/patient management.	
Infection lasting more than 30 days or persistent cycles of recurrence.	
Failure to respond as expected to antimicrobial therapies (e.g. systemic antibiotics and/or topical antimicrobials/antiseptics. Infection may re-emerge when treatment is completed.	
Signs of local infection (e.g. erythema, oedema, serous effusion, new areas of breakdown, pain, etc.).	
Persistent or prolonged (low-level) inflammation.	
Soft tissue deterioration despite antibiotic treatment and/or surgical debridement.	
A shiny, slimy layer on a non-healing wound surface that persists despite autolytic or enzymatic debridement but can be removed easily with physical removal techniques such as cleansing or mechanical debridement. The surface reforms quickly (1-2 days) in the absence of frequent intervention (e.g. cleansing, debridement).	

Presence of slough or fibrin/fibrin coating.	
Poor quality granulation tissue (e.g. discoloured, friable, fragile or hypergranulation).	
Tunnelling (along tissue structures, degradation along solid structures such as tendon or bone) and/or undermining (erosion of the subcutaneous layer under the wound edge).	

Comments (if any): _____

To be copied on to study site headed paper

Diabetes Day Centre,
Hospital 5,
St. James's Hospital,
James's St.,
Dublin D08 TYF3
Tel: 01 428 4437

Date of study visit

Dear Care provider,

We would like to inform you that the bearer of this letter participated in a clinical study entitled:

“A clinical study to validate signs and symptom reported in the literature to be indicative of biofilm in chronic wounds against a gold standard biopsy in a sample of patients with diabetic foot ulceration”. at the Diabetes Day Centre, St. James's Hospital on the above date.

As part of the procedure a punch biopsy 4mm in width x up to 5mm in depth was taken from the edge of the bearer's study wound.

We would ask that you give special attention to the biopsy site while providing wound care and to contact us directly at the telephone number above should you have any concerns.

Sincerely yours,

Dr. Niamh Phelan MB BCh NUI FRCPI MD

Investigative site: _____

Patient participant name	DOB	Date recruited	Study ID #	Comments (e.g. failed eligibility criteria, withdrew consent, completed)
			001	
			002	
			003	
			004	
			005	
			006	
			007	
			008	
			009	
			010	
			011	
			012	
			013	
			014	
			015	
			016	
			017	
			018	
			019	
			020	



Marsh Ireland Brokers Ltd
Charlotte House
Charlemont Street
Dublin 2
Ireland

D02 NV26
Tel: 01 604 8100 www.marsh.ie

4th October 2024

Private & Confidential

To Whom It May Concern

CONFIRMATION OF INSURANCE – University of Galway and/or subsidiary and/or associated companies

As requested by the above client, we are writing to confirm that we act as Insurance Brokers to the client and that we have arranged insurance(s) on its behalf as detailed below with insurers who are authorised to conduct insurance business in Ireland by their respective regulatory authority(ies).

LIFE SCIENCE POLICY

INSURER:	Newline Europe Versicherung AG
POLICY NUMBER:	NDL23038000A/B/C
PERIOD OF INSURANCE:	1st October 2024 to 30th September 2025
LIMITS:	€15,000,000 in the aggregate any one period of insurance.
EXCESS:	€5,000 each and every claim

The above Policy provides the following covers:

No Fault Compensation for Human Clinical Trials, Legal Liability for Human Clinical Trials, Errors & Omission Liability, Products Liability and Medical Malpractice Liability.

Appendices

We have placed the insurance which is the subject of this letter after consultation with the client and based upon the client's instructions only. Terms of coverage, including limits and deductibles, are based upon information furnished to us by the client, which information we have not independently verified.

Marsh Ireland Brokers Limited, trading as Marsh Ireland, Bowring Marsh, Charity Insurance, Echelon Claims Consultants, Guy Carpenter & Company, ILCS, Insolutions, Lloyd & Partners, Marsh Aviation Consulting, Marsh Claims Management Services, Marsh Claims Solutions, Marsh Specialty, Marsh Reclaim, and Marsh Risk Consulting, is regulated by the Central Bank of Ireland.

Marsh Ireland, Bowring Marsh, Charity Insurance, Echelon Claims Consultants, Guy Carpenter & Company, ILCS, Insolutions, Lloyd & Partners, Marsh Aviation Consulting, Marsh Claims Management Services, Marsh Claims Solutions, Marsh Specialty, Marsh Reclaim, and Marsh Risk Consulting are trading names of Marsh Ireland Brokers Limited. Marsh Ireland Brokers Limited is a private company limited by shares registered in Ireland under company number 169458. VAT Number IE 6569458D. Registered Office: Charlotte House, Charlemont Street, Dublin 2, Ireland, D02 NV26. Directors: A J Croft, P F Doyle, P G Dromgoole (British), J C Grogan, E Kelly, D McGovern, C Nelson (British) and C. Ryan.



A business of Marsh McLennan

This letter is issued as a matter of information only and confers no right upon you other than those provided by the policy. This letter does not amend, extend or alter the coverage afforded by the policies described herein. Notwithstanding any requirement, term or condition of any contract or other document with respect to which this letter may be issued or pertain, the insurance afforded by the policy (policies) described herein is subject to all terms, conditions, limitations, exclusions and cancellation provisions. Limits shown may have been reduced by paid claims.

We express no view and assume no liability with respect to the solvency or future ability to pay of any of the insurance companies which have issued the insurance(s).

We assume no obligation to advise yourselves of any developments regarding the insurance(s) subsequent to the date hereof. This letter is given on the condition that you forever waive any liability against us based upon the placement of the insurance(s) and/or the statements made herein with the exception only of wilful default, recklessness or fraud.

This letter may not be reproduced by you or used for any other purpose without our prior written consent.

This letter shall be governed by and shall be construed in accordance with Irish law.

Yours sincerely,

A handwritten signature in blue ink, appearing to read "Graham O'Hehir", is written over a light blue horizontal line.

Graham O'Hehir

Appendices

Vice President
Marsh Ireland Brokers Ltd
Direct Dial: 01 604 8276
E-mail: Graham.OHehir@marsh.com

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Merlin Park University Hospital Saolta GALWAY
Grúpa Ollscoile Cúram S16inte
University Health Care Group



Mheirlinne
UNIVERSITY HOSPITALS

Clinical Research Ethics Committee

Room 2
2nd Floor
HR Building

Merlin Park Hospital Galway.

4th June, 2024.

Mr. John Ivory
Room 234
School of Nursing & Midwifery
Aras Moyola
University of Galway
Galway
H91 TK33.

Ref: C.A. 3232 A Clinical Study to validate signs and symptoms reported in the literature to be indicative of biofilm in chronic wounds against a gold standard biopsy in a sample of patients with diabetic foot ulceration

Dear Mr. Ivory,

Thank you for your revised submission and clarification as requested by the Clinical Research Ethics Committee following its meeting on the 8th May, 2024. I am happy to confirm that the above study is now fully approved.

The date of this letter is the date of authorization of the study.

Please keep a copy of this signed approval letter in your study master file for audit purposes. The study must be carried out in accordance with General Data Protection Regulation and Health Research Regulation 2018.

You should note that ethical approval will lapse if you do not adhere to the following conditions:

Appendices

1. Submission of an Annual Progress Report/Annual Renewal Survey (due annually from the date of this approval letter). We would encourage you to keep note of this date as the CREC will not issue a reminder.
2. Report unexpected adverse events, serious adverse events or any event that may affect ethical acceptability of the study

Ospidéal na h-Ollscoile, Péirc Mheirlinne, MERLIN PARK UNIVERSITY HOSPITAL,
Galway, Ireland. Tel: 00 353 (0)91 757631
3. Submit any change to study documentation (minor or major) to CREC for review and approval. Amendments must be submitted on an amendment application form and revised study documents must clearly highlight the changes and contain a new version number and date. Amendments cannot be implemented without written approval from CREC.
4. Notify CREC of discontinuation of the study
5. Submit an End of Trial Declaration Form and Final Study Report/Study Synopsis when the study has been completed.

This application has been reviewed from an Ethical perspective. It remains the responsibility of the Principal Investigator to ensure that data processes are compliant with National Data Protection Regulations and local policies.

Yours sincerely,



Professor Peter A. McCarthy

Chair CREC Galway University Hospitals

Emeritus Professor, University of Galway

Consultant Radiologist, Bon Secours Hospital and Galway Clinic formerly

Established Professor and Head of Radiology/ Consultant Radiologist NUI Galway/

Galway University Hospitals.

Appendices

c.c. Professor Sean Dinneen, Senior Lecturer, School of Medicine, University of Galway. Professor Georgina Gethin, Senior Lecturer, School of Nursing & Midwifery, Room 407, Aras Moyola, University of Galway.



Merlin Park University Hospital  Saolta
HOSPITALS

Grúpo Ollscoile Cúram Sláinte

University Health Care Group



Ospidéal na h-Ollscoile, Páirc Mheirlinne

GALWAY UNIVERSITY

Clinical Research Ethics Committee

Room 2

2nd Floor

HR Building

Merlin Park Hospital Galway.

29th November 2024.

Mr. John Ivory

Room 234

School of Nursing & Midwifery

Aras Moyola

University of Galway

Galway

H91 TK33.

Ref: C.A. 3232

A Clinical Study to validate signs and symptoms reported in the literature to be indicative of biofibrin in chronic wounds against a gold standard biopsy in a sample of patients with diabetic foot ulceration

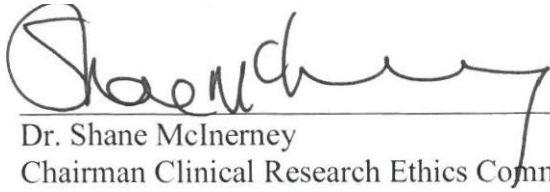
Amendment submitted 8th August, 2024 — Revised Protocol, Patient Information Leaflet, Clinician Assessment Patient Information Leaflet and Letter to Care Provider

Dear Mr. Ivory,

I have considered the above amendment, and I wish to confirm Chairman's approval to proceed.

Chairman's approval is normally ratified at the next Clinical Research Ethics Committee meeting. If any issues with your application are identified at the meeting we will contact you again.

Yours sincerely,



Dr. Shane McInerney
Chairman Clinical Research Ethics Committee

Chairman

Clinical Research Ethics Committee

Consultant General Adult Psychiatrist

Honorary Senior Lecturer in Psychiatry, University of Galway

National Clinical Lead for Self Harm and Suicide Related Ideation.

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