EUROPEAN TISSUE REPAIR SOCIETY (ETRS) ANNUAL MEETING

ADVANCES IN TISSUE REPAIR AND REGENERATION – TOWARDS NOVEL THERAPEUTICS

3rd - 5th September 2025

Cardiff, Wales

WECOME MESSAGE

It is with great pleasure that we say "*Croeso*!" and welcome you all to Cardiff for the ETRS 2025 Conference. We are delighted to host you in our historic city, the capital of Wales, especially as ETRS Conferences have been a longstanding means of disseminating research and collaboration for numerous researchers and clinicians from Cardiff over many years.

The theme for the Conference is '*Advances in Tissue Repair and Regeneration – Towards Novel Therapeutics*', which is centred around a focus on our current knowledge and understanding of the mechanisms underlying normal, pathological and privileged healing scenarios to develop new therapies to aid the management and treatment of clinical situations associated with dysfunctional repair. Our renowned speakers will cover a wide range of topics relevant to wound healing and regenerative medicine, from basic sciences through to therapy translation for clinical use.

Our Conference venue, the Cardiff University Centre for Student Life, is at a central location easily accessible from most local hotels, as is our Conference Dinner at Cardiff Castle. Such landmarks are at the heart of our city that presents a vibrant and diverse atmosphere for people to enjoy, which we highly recommend you take time to explore.

So, please explore our Programme and take advantage of the networking opportunities available with experts in wound healing, regenerative medicine and therapy translation, via the excellent research Programme and social events we have arranged.

We hope the experience will be an enjoyable and enriching experience for all!

Best regards,

ETRS 2025 Organising Committee.

INDEX

CONFERENCE VENUES

EARLY CAREER RESEACHER PRE-CONFERENCE

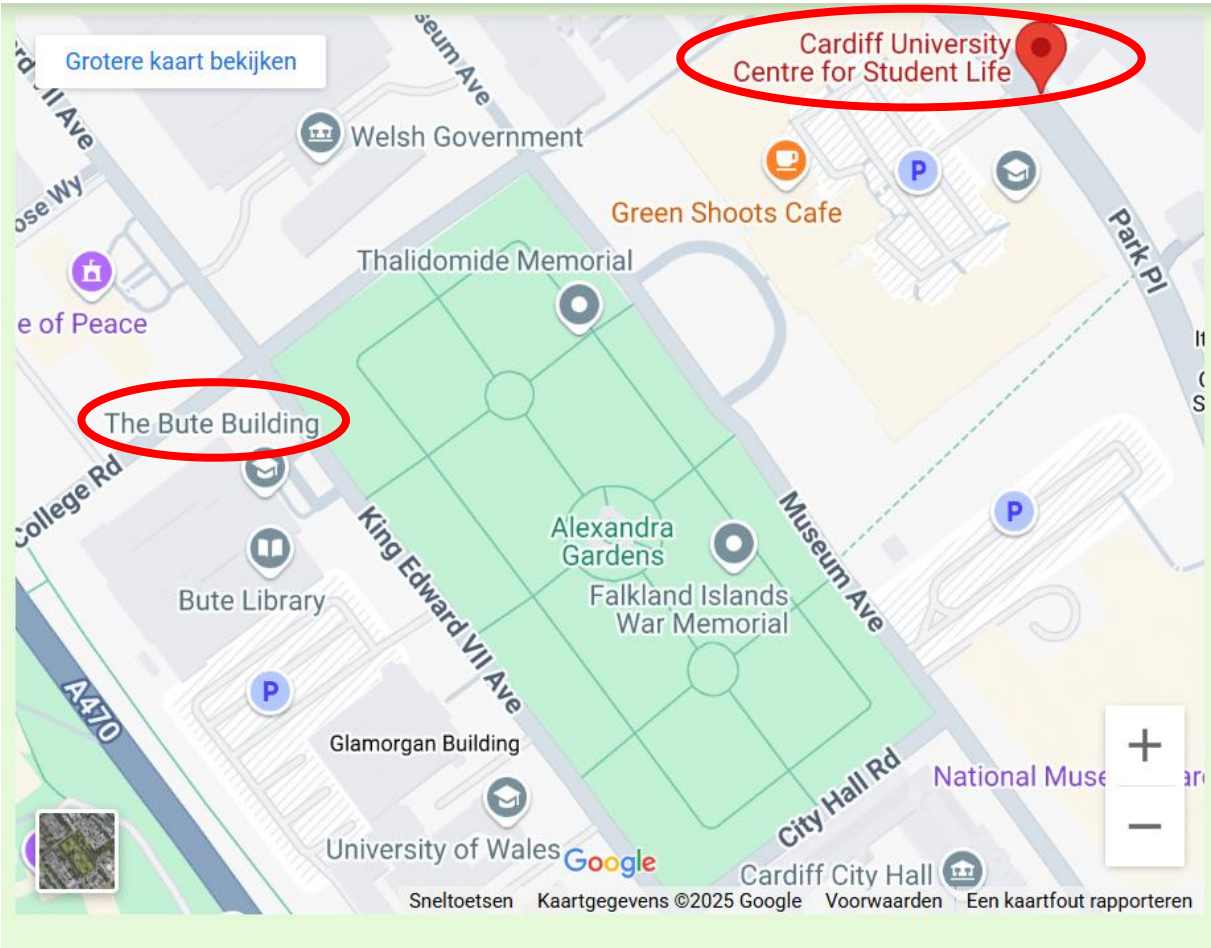
TUESDAY 2nd SEPTEMBER 2025

VENUE: **Bute Building, Cardiff University, King Edward VII Avenue, Cardiff. CF10 3NB.**

ETRS ANUUAL MEETING

WEDNESDAY 3rd – FRIDAY 5th SEPTEMBER 2025

VENUE: **3rd and 4th Floors, Centre for Student Life, Cardiff University, Park Place, Cardiff. CF10 3BB.**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Falkland Islands
War Memorial
Museum Ave
Bute Library
King Edward VII Ave
A470
Glamorgan Building
City Hall Rd
University of Wales
Google
Cardiff City Hall
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Voorwaarden
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WIFI ACCESS

The free wireless network, **'Eduroam'**, should be accessible across the Cardiff University campus, for those delegates with access to it.

WIFI connection to mobile devices for delegates without Eduroam access:

1. Select 'CU-Wireless' from your device's list of wireless network services.

If 'not listed, find the network by selecting 'Other' and searching for 'CU-Wireless'.

At the CU-Wireless WIFI page, select 'Cardiff University Conferences'.

2. If you are not auto-directed to this page, please go to 'Guest.cardiff.ac.uk'.

Enter the Conference ID: **CONF2025**, followed by the Password: **811434**.

At the CU-Wireless Conference WIFI page, complete the form with your name, email address, and phone number.

Select 'I accept the terms of use' and 'Register' to continue.

The system will send your username and password to the email address provided for reference.

Select 'Log in' to continue.

Your device should automatically log on to the university CU-Wireless network.

To connect an additional device to the Conference ID:

1. Select 'CU-Wireless' from the list of networks available.
2. At the CU-Wireless WiFi page, select 'Cardiff University Conferences'.
3. At the CU-Wireless WiFi page, select 'Sign in' at the bottom of the page next to 'Already have an account'.
4. Use the Username and Password provided within the email sent earlier.
5. The device will automatically log on to the university CU-Wireless network.

Should you encounter any problems connecting to the network, please contact the Conference Registration Desk.

SOCIAL PROGRAMME

WELCOME DRINKS RECEPTION

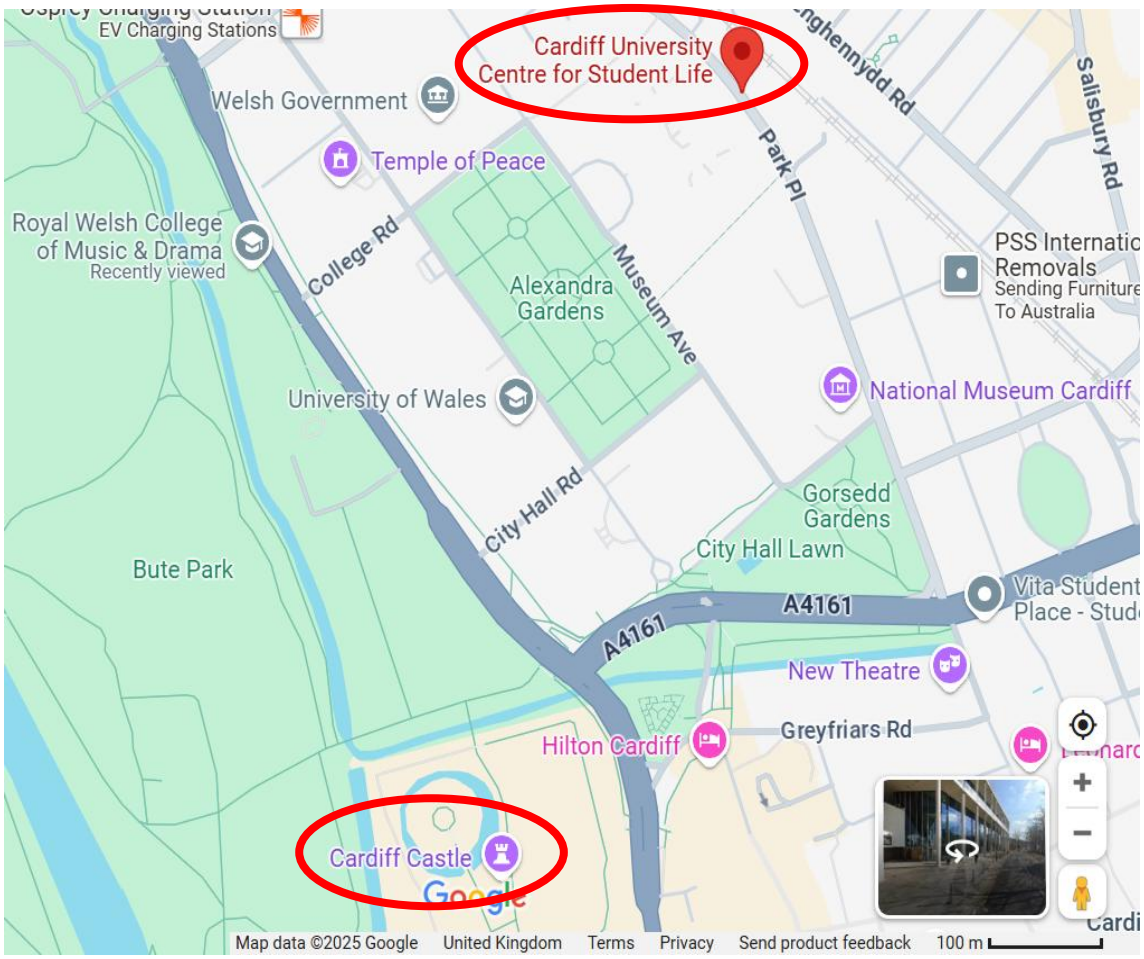
WEDNESDAY 3rd SEPTEMBER 2025

VENUE: **3rd and 4th Floors, Centre for Student Life, Cardiff University, Park Place, Cardiff. CF10 3BB.**

CONFERENCE DINNER

THURSDAY 4th SEPTEMBER 2025

VENUE: **Cardiff Castle, Castle Street, Cardiff. CF10 3RB.**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Royal Welsh College
of Music & Drama
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Removals
Sending Furniture
To Australia
University of Wales
National Museum Cardiff
City Hall Rd
Gorsedd
Gardens
City Hall Lawn
Bute Park
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New Theatre
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VISITING CARDIFF AND WALES

There is plenty to do in Cardiff city centre, as well as further afield.

You can find a great compilation of things to see and do around Cardiff and across Wales, as well as places to eat and drink, places to stay, and other information, at the Visit Cardiff and Visit Wales websites.

Amongst the unmissable sites of Cardiff, is Cardiff Castle and its grounds. The Castle is surrounded by the large and very well-maintained Bute Park to the north, and provides a beautiful view of the city centre to the south, all the way to Cardiff Bay. Entry to the castle grounds is typically free (unless there is an event taking place), but you will need a ticket to visit the walls, the fort, and the gothic palace. We will also be visiting the Castle for the Conference Dinner.

The pedestrianised city centre is very lively, bursting with restaurants, bars, and shops, and a couple of museums, including the National Museum of Wales (National Museum). There is also the National Museum of Welsh Life, just outside Cardiff (National Museum of History).

Cardiff is also known as the *City of Arcades*, due to the several Victorian and Edwardian arcades that nestle amongst the city centre streets. The Principality Stadium is home to the Welsh national rugby team, is also impressively set in the city centre.

With a short bus or train trip from the city centre, you can also reach Cardiff Bay, home of the Welsh Parliament Building (Senedd Cymru), the Millenium Centre (Wales Millennium Centre), The Pierhead (historic Grade 1 listed building, now a museum), and the Cardiff Bay barrage that copes with impressive ~10m tides and prevents Cardiff from flooding.

Cardiff Food/Drink Guide

With the Conference venue, social events and local hotels being so close to the city centre, there are a wide range of bars, traditional Cardiff pubs, nightclubs and restaurants within walking distance, to cater for all tastes and budgets. Many of these are located on *St Mary's Street, The Hayes, Castle Street, Westgate Street and Church Street.* You can find information on many places to eat and drink at the Visit Cardiff website.

LOCAL ORGANISING COMMITTEE

Ryan Moseley, ETRS Secretary

Conference Chair

Phil Stephens, ex-ETRS President

Bouke Boekema, ETRS President

Peter Moortgat, ETRS Treasurer

Tim Bowen

Helen Brown

Oommen Oommen

Andrew Quantock

Christophe Egles, Conference

Chair ETRS 2026, University of Rouen

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Ryan Moseley, ETRS Secretary

Conference Chair

Kiranjit Bains

Helen Brown

Sian Morgan

Oommen Oommen

Maya Ezzo

Adriana Panayi

SCIENTIFIC COMMITTEE

Ryan Moseley - *Cardiff University, UK*

Kiranjit Bains - *Cardiff University, UK*

Bouke Boekema - *Alliance of Dutch Burn Care, The Netherlands*

Tim Bowen - *Cardiff University, UK*

Helen Brown - *Cardiff University, UK*

Shane Browne - *Royal College of Surgeons in Ireland, Ireland*

Willeke Daamen - *Radboud University Medical Centre, The Netherlands*

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Maya Ezzo - *Pasteur Institute, France*

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Andrew Leask - *University of Saskatchewan, Canada*

Sian Morgan - *Cardiff University, UK*

Oommen Oommen - *Cardiff University, UK*

Adriana Panayi - *Trauma Centre Ludwigshafen, Germany*

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Hans Smola - *University of Cologne, Germany*

Phil Stephens - *Cardiff University, UK*

CONFERENCE SPONSORS

Early Career Researcher Pre-Conference

SCIENTIFIC PROGRAMME

DAY 1	**Wednesday 3rd September**	
08:00-10:00	*Delegate Registration and Poster Placement*	
08:45-9:45	*ETRS Board Meeting (**Room 2.25/2.26**), 2nd Floor, Centre for Student Life, Cardiff University*	
10:00-10:15	*Welcome Address*	
SESSION 1: 3D Bioprinting, Biomaterials and Bioengineering		
10:15-12.30	Chairs	*Christophe Egles, University of Rouen, France* *Willeke Daamen, Radboud University Medical Centre, The Netherlands*
Keynote Lecture **10:15-11:05**	Modifying Healing Using Soft Matter. **Professor Liam Grover, University of Birmingham, UK**	
11:05-11:30	Smart Hyaluronan Hydrogels for Inducing Pro-Healing Macrophage Responses and Neuronal Regeneration. **Dr Oommen Oommen, Cardiff University, UK**	
11:30-11:45	**O1.** Engineering Hyaluronic Acid-Based Bioink for Ultra-Fine 3D Bioprinting to Develop *in Vitro* Models. **Dr Oommen Varghese, Uppsala University, Sweden**	
11:45-12:00	**O2.** Support Matrix Influences on Printing Resolution in a 3D Bioprinted Human Skin Model. **Saskia Fogg, University of Birmingham, UK**	
12:00-12:15	**O3.** Human Skin Substitute With Restored Innervation, Vasculature, and Hair Follicles for Full-Thickness Wound Treatment. **Mahrukh Riaz, University of Zurich, Switzerland**	
12:15-12:30	**O4.** Adjusting the Handling of a Cell Free Hydrogel System Based on Wharton's Jelly Extracellular Matrix to Optimise Facial Nerve Regeneration. **Dr Elise Krawiec, University of Reims Champagne-Ardenne, France**	
12:30-13:30	*Lunch*	

SESSION 2: Stem Cells and Regenerative Medicine		
13:30-15:30	Chairs	*Andrew Quantock, Cardiff University, UK* *Shane Browne, Royal College of Surgeons in Ireland, Ireland*
Keynote Lecture **13:30-14:20**	Mesenchymal Stromal Cells: Repairing Tissues and Reputations. **Professor Paul Genever, University of York and Mesenbio, UK**	
14:20-14:45	Engineering Eye-Like Organoids and Tissues from Human iPS Cells: Applications for Regenerative Medicine. **Dr Laura Howard, Cardiff University UK**	
14:45-15:00	**O5.** Generation of a Stem Cell-Derived Cell Therapy Product for Huntington's Disease. **Dr Mariah Lelos, Cardiff University, UK**	
15:00-15:15	**O6.** Corneal Epithelial Wound Healing by Purified Components from Immortalized Stem Cells From Human Exfoliated Deciduous Teeth. **Professor Yuji Teramura, National Institute of Advanced Industrial Science and Technology, Japan**	
15:15-15:30	**O7.** Development of Extracellular Matrix Protein-Decorated Hydrogels for Vascularisation. **Joanne Chang, Royal College of Surgeons in Ireland, Ireland**	
15:30-16:00	*Coffee Break*	
16:00-17:00	**Charles Lapière Memorial Lecture**	
	Chairs	*Bouke Boekema, Alliance of Dutch Burn Care, The Netherlands* *Ryan Moseley, Cardiff University, UK*
New Bioengineering-based Approaches for Therapeutics and Biosensing in Tissue Repair. **Dame Professor Molly Stevens, Oxford University, UK**		
17:00-19:00	*Poster Session 1 & Welcome Drinks Reception* *Centre for Student Life, Cardiff University*	

DAY 2	**Thursday 4th September**	
SESSION 3: Infection, Inflammation and Wound Healing		
08:30-10:05	Chairs	*Sandra Franz, Leipzig University, Germany* *Helen Brown, Cardiff University, UK*
Keynote Lecture **08:30-09:20**	Derailed Cellular Choreography in the Skin Ulcer Niche: Roadblocks to Repair. **Dr Jenna Cash, University of Edinburgh, UK**	
09:20-09:35	**O8.** Iron-Induced Changes in Dermal Fat-Fibroblast-Signalling Axis Drive Disease progression in Lipodermatosclerosis. **Dr Marta Torregrossa, Leipzig University, Germany**	
09:35-09:50	**O9.** Identification and Characterisation of AKR101 as Potential New Therapeutic for Chronic Wounds. **Dr Petra Döerfler, Akribes Biomedical, Austria**	
09:50-10:05	**O10.** A Clinical Trial Examining *Ficus Septica* Latex for the Treatment of Small Cutaneous Ulcers in Papua New Guinea. **Dr Tom Prescott, Royal Botanical Gardens Kew, UK**	
10:05-10:30	*Coffee Break*	
SESSION 4: Scarring and Wound Regeneration		
10:30-12:30	Chairs	*Andrew Leask, University of Saskatchewan, Canada* *Phil Stephens, Cardiff University, UK*
Keynote Lecture **10:30-11:20**	Exploiting the Hair Follicle to Promote Skin Healing and Remodel Scar Tissue. **Dr Claire Higgins, Imperial College London, UK**	
11:20-11:45	Can we Modify the Hyaluronan Matrix to Promote Renal Recovery After Injury? **Dr Soma Meran, Cardiff University UK**	
11:45-12:00	**O11**. A Novel Population Health-Based Pipeline to Screen for Scarring Genes Reveals a Role for LGR4 in Skin Repair. **Dr Oscar Peña Cabello, University of Bristol, UK**	
12:00-12:15	**O12.** CCN3-Based Peptide BLR-200 Has Anti-Fibrotic Properties in Systemic Sclerosis. **Professor Andrew Leask, University of Saskatchewan, Canada**	
12:15-12:30	**O13:** Development of a Flightless I Neutralising Antibody Therapy for the Treatment of Burns. **Dr Xanthe Venn, University of South Australia, Australia**	
12:30-13:30	*Lunch and Poster Session 2*	

SESSION 5: Drug Development for Wound Healing / QBiotics Symposium		
13:30-15:20	Chairs	*Tim Bowen, Cardiff University, UK* *Oommen Oommen, Cardiff University, UK*
Keynote Lecture **13:30-14:00**	EBC-1013 From Lab to Clinic: The Scientific, Technical and Commercial Challenges in Developing a Novel Wound Healing Drug. **Dr Paul Reddell, QBiotics Group, Australia**	
14:00-14:20	The Application of the Epoxytigliane, EBC-1013, in the Treatment of Infected Wounds: Defining the Orthogonal Mechanisms of Action. **Professor David Thomas, Cardiff University, UK**	
14:20-14:40	Investigating Epoxytigliane-Mediated Immunomodulatory Processes in the Context of Chronic Wound Healing. **Dr Jason Cullen, QBiotics Group, Australia**	
14:40-15:00	Chronic Wound Fibroblast Responsiveness to Epoxytigliane-Stimulated Repair Mechanisms is Dependent on Fibroblast Kinome Profiles. **Dr Emma Woods, QBiotics Group, Australia**	
15:15-15:20	Epoxytigliane Stimulation of Keratinocyte Wound Healing Responses Promote Enhanced Skin Re-Epithelialisation. **Professor Ryan Moseley, Cardiff University, UK**	
15:20-15:25	Wounds in Cardiff: 20 Years On. **Professor Keith Harding, Cardiff University, UK**	
15:25-15:45	*Coffee Break*	
SESSION 6: ETRS Young Investigator Award / WHS Session		
15:45-18:15	Chairs	*Oommen Oommen, Cardiff University, UK* *Bouke Boekema, Alliance of Dutch Burn Care, The Netherlands*
15:45-16:00	**Y1.** Understanding Confined Functions of Neutrophils in the Pathophysiology of Diabetic Wound Healing. **Jonathan Kessler, Leipzig University, Germany**	
16:00-16:15	**Y2.** Synergism of Endothelial Cell Death and Fibroblast Senescence in Diabetic Angiopathy. **Yongfang Wang, Ulm University, Germany**	
16:15-16:30	**Y3.** Identifying Novel Wound Extravasation Genes Using a Drosophila-to-Murine Pipeline. **Terrance Trinca, University of Bristol, UK**	
16:30-16:45	**Y4.** Proteomic Profiling of Platelet-Rich Plasma to Predict Therapy Outcomes in Complex Wounds. **Anna Buisan-Farre, Central University of Catalonia, Spain**	
16:45-17:00	**Y5.** Alginate-Based Hydrogels Loaded With Human Beta-Defensin-2 Enhance Healing of Infected and Non-Infected Wounds in a Diabetic Mouse Model. **Jessica Da Silva, University of Coimbra, Portugal**	
17:00-17:15	**Y6.** The Immunomodulator Soluble CD83 Ameliorates Wound Healing Quality in a Murine Pressure Ulcer Mode by Inducing Tissue Repair Macrophages. **Fabian Stritt, University Hospital Erlangen, Germany**	

17:15-17:30	**Dr Suwelack Corporate Foundation Head of Consortium Speech. Professor Adrian Dragu.** **Dr Suwelack Corporate Foundation Young Investigator Award Winner:** Effect of Hyaluronan in Collagen Biomaterials on Human Macrophages and Fibroblasts *In Vitro*. **Nancy Avila-Martinez, Radboud University Medical Centre, The Netherlands**
17:30-17:45	**WHS YIA Winner, 2025:** Statin-Cyclodextrin Hydrogels Accelerate Healing In Multiple Pre-Clinical Wound Models. **Veronika Jurczuk, University of Miami, USA**
17:45-18:00	**WHS Mid-Career Researcher 1:** Endothelial Metallothionein is Epigenetically Regulated and Indispensable for Diabetic Ischemic Tissue Rescue Following VEGF Therapy. **Dr Kanhaiya Singh, University of Pittsburgh, USA**
18:00-18:15	**WHS Mid-Career Researcher 2:** Role of Fibroblast Energy Metabolism and Exosome Signalling in Regulating Wound Healing and Fibrosis. **Dr Swathi Balaji, Baylor College of Medicine, USA**
18:15-19:00	**ETRS General Assembly**
19:00-23:00	*Conference Dinner, Cardiff Castle*

DAY 3	Friday 5th September	
SESSION 7: Wound Healing Therapy Translation, Regulation and Public Perception (Workshop)		
08:30-10:35	Chairs	*Paul Reddell, QBiotics Group, Australia* *Ryan Moseley, Cardiff University, UK*
08:30-08:45	Introduction to Workshop. **Dr Paul Reddell, QBiotics Group, Australia**	
08:45-09:05	University Spin-Out of Biomedical Research: From Academia to SME. **Professor Paul Genever, University of York and Mesenbio, UK**	
09:05-09:25	What Does Medtech Look for in Terms of the Development and Commercialisation of New Wound Healing Therapies? **Dr Alan Horner, Horner Scientific Consulting, UK**	
09:25-09:45	Regulatory Considerations Surrounding Regenerative Medicine Therapy Translation and Commercialisation. **Dr Lindsay Davies, NextCell Pharma, Sweden**	
09:45-10:05	Shifting Perceptions: Comparative Perspectives on Regenerative Medicine Development. **Dr Maki Umemura, Cardiff University, UK**	
10:05-10:35	Open Forum Discussion	
10:35-11:00	*Coffee Break*	
SESSION 8: Matrix Modulation For Tissue Repair		
11:00-12:40	Chairs	*Sian Morgan, Cardiff University, UK* *Tim Bowen, Cardiff University, UK*
Keynote Lecture **11:00-11:50**	Systemically Administered, Target-Specific Therapies for Tissue Repair. **Professor Tero Järvinen, Tampere University, Finland**	
11:50-12:15	Are Heparan Sulfates the Lead to Reach the Grail of Tissue Regeneration? **Professor Denis Barritault, OTR3, France**	
12:15-12:40	Type III Collagen: Modulator of Biochemical, Biomechanical, and Biophysical Dynamics in Tissue Repair. **Professor Susan Volk, University of Pennsylvania, USA**	
12:40-13:30	*Lunch*	

SESSION 9: Exploiting Biologics For Enhanced Healing		
13:30-15:30	Chairs	*Kiranjit Bains, Cardiff University, UK* *Phil Stephens, Cardiff University, UK*
Keynote Lecture **13:30-14:20**	Decoding the RNA Regulome of Human Skin Wound Healing – Towards Future Wound Care. **Dr Ning Xu Landén, Karolinska Institute, Sweden**	
14:20-14:45	MicroRNAs as Disease Sentinels and Therapeutic Targets in Patients with Kidney Disease. **Professor Tim Bowen, Cardiff University, UK**	
14:45-15:00	**O14.** Topical Exosomes Mediate Wound Healing Benefit Via Modulation of Innate Immune Cellular Milieu. **Dr Piul Rabbani, New York University School of Medicine, USA**	
15:00-15:15	**O15.** Targeting PTP1B Enhances Diabetic Wound Healing by Modulating Macrophage Polarization Via HO-1 Pathway. **Dr Ermelindo Leal, University of Coimbra, Portugal**	
15:15-15:30	**O16.** Soluble CD83 Enhances Intestinal Wound Healing in DSS-Induced Colitis. **Phillipp Beck, University Hospital Erlangen, Germany**	
15:30-15:45	Awards and Closing Remarks	
CLOSE		

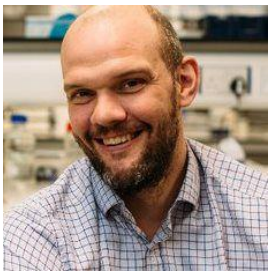
INVITED SPEAKERS

Invited Speakers - ETRS 2025

Charles Lapière Memorial Lecture

Dame Professor Molly Stevens

University of Oxford, UK



Professor Liam Grover

University of Birmingham, UK

Professor Paul Genever

University of York, UK

Dr Jenna Cash

University of Edinburgh, UK

Dr Claire Higgins

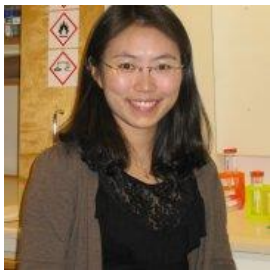
Imperial College London, UK

Dr Paul Reddell

QBiotics Group, Australia

Professor Tero Järvinen

Tampere University, Finland



Dr Ning Xu Landén

Karolinska Institute, Sweden

Dr Oommen Oomen

Cardiff University, UK

Dr Laura Howard

Cardiff University, UK

Dr Soma Meran

Cardiff University, UK

Professor David Thomas

Cardiff University, UK

Dr Jason Cullen

QBiotics Group, Australia

Dr Emma Woods

QBiotics Group, Australia

Professor Ryan Moseley

Cardiff University, UK

Dr Alan Horner

Horner Scientific Consulting, UK

Dr Lindsay Davies

NextCell Pharma, Sweden

Dr Maki Umemura

Cardiff University, UK

Professor Denis Barritault

OTR3, France

Professor Susan Volk

University of Pennsylvania, USA

Professor Tim Bowen

Cardiff University, UK

SELECTED ORAL PRESENTATION ABSTRACTS

O1. ENGINEERING HYALURONIC ACID-BASED BIOINK FOR ULTRA-FINE 3D BIOPRINTING TO DEVELOP *IN VITRO* MODELS

Varghese OP[1], Tavakoli S[1]

[1]Uppsala University, Sweden.

Background: 3D bioprinting is an innovative technology bridging tissue engineering and additive manufacturing, enabling the precise, layer-by-layer deposition of bioinks to fabricate implantable tissues and advanced biological models. Despite notable progress, developing an ideal bioink with balanced biological, physical, and mechanical properties remains a challenge. To address this challenge, this study aims to develop a shear-thinning, disulfide-crosslinked hyaluronic acid (HA) hydrogel to create a stable and mechanically robust bioink for advanced 3D bioprinting applications.

Methods/Results: To develop HA-based biomaterial for bioprinting, we modified less than 10% of the carboxylic acid backbone with cysteine units. This allowed for the formation of a dynamic, disulfide-crosslinked hydrogel through a simple pH adjustment. To overcome the characteristically slow gelation at physiological pH, we introduced potassium iodide as a novel catalyst. The catalyst significantly accelerated gelation in a concentration-dependent manner and also acted as a radical scavenger. The optimized hydrogel's printability was confirmed by successfully extruding it through fine-gauge (32G, 108 µm inner diameter) needles and demonstrating its ability to encapsulate cells within complex 3D structures. The disulfide-crosslinked HA hydrogel successfully encapsulated human mesenchymal stem cells (hMSCs) and chondrocytes, maintaining their viability and enabling interaction within the matrix. The disulfide linkages allowed us to control the degradation rate based on the cellular environment and the density of encapsulated cells. Using a 32G needle, we achieved high-resolution, layer-by-layer 3D bioprinting of intricate structures. Co-cultures of hMSCs and chondrocytes within the printed constructs facilitated the investigation of cell-cell interactions, migration, and the dynamic behaviour of cells in a 3D environment.

Conclusions: Cysteine-modified HA hydrogels offer a promising solution for advanced 3D bioprinting, especially for high-precision applications using ultra-fine needles. The ability to achieve gelation with a single gel component represents a significant step toward creating sophisticated *in vitro* models for tissue engineering and regenerative medicine.

O2. SUPPORT MATRIX INFLUENCES ON PRINTING RESOLUTION IN A 3D BIOPRINTED HUMAN SKIN MODEL

Fogg SM[1,2], Perez-Esteban P[1], Metcalfe A[1], Le H[2], Grover LM[1]

[1]University of Birmingham, UK.

[2]Bayer AG, Germany.

Background: Suspended layer additive manufacturing (SLAM) is a bioprinting technique that uses fluid gels as a temporary support matrix to enable the extrusion of soft hydrogel-based bioinks into complex, functional three-dimensional (3D) tissue structures. It presents a promising strategy for engineering human skin equivalents (HSEs) that replicate the structure and function of native skin. These constructs may improve treatment outcomes for chronic or non-healing wounds caused by burns, trauma, or disease. While most research has focused on optimising bioink formulation, extrusion pressure, and nozzle geometry to improve print resolution, the influence of the support matrix remains underexplored. This study aims to characterise and optimise fluid gel support matrices to improve print resolution and demonstrate SLAM's application in fabricating a tri-layered HSE.

Methods: Agarose fluid gels with varying concentrations and particle sizes were rheologically characterised. Composite hydrogels of pectin and collagen were used as bioinks, each combined with one of three human cell types, epidermal keratinocytes, dermal fibroblasts, or adipose-derived stem cells, to mimic the three layers of skin. Single filaments of each bioink were printed into each agarose sample and assessed for print resolution and fidelity using optical microscopy. Finally, a tri-layered HSE was printed using optimised conditions.

Results: The support matrix is one of the critical determinants of print resolution; increasing the concentration and reducing the particle size significantly improved resolution and print fidelity. The tri-layered structure was successfully fabricated using optimised gel conditions.

Conclusions: This study demonstrates that tuning the physical properties of the support matrix can enhance SLAM resolution. While further validation of long-term cell viability is needed, these findings support SLAM as a valuable tool for producing multilayered, clinically relevant skin constructs.

O3. HUMAN SKIN SUBSTITUTE WITH RESTORED INNERVATION, VASCULATURE, AND HAIR FOLLICLES FOR FULL-THICKNESS WOUND TREATMENT

Riaz M[1,2], Ben Nejma A[1,2], Iqbal MZ[1,2], Pontiggia L[1,2], Moehrlen U[1,2], Klar AS[1,2], Biedermann T[1,2]

[1]University Children's Hospital Zurich, Switzerland.

[2]University of Zurich, Switzerland.

Background: Deep skin wounds are difficult to treat and rarely heal without surgical intervention. Although skin grafts composed of keratinocytes and fibroblasts provide coverage, they lack vascular networks, nerve supply, and skin appendages, such as hair follicles, limiting their functionality and sensory recovery. This study aimed to develop a human skin substitute that is prevascularized, preinnervated, and hair follicle spheroid-integrated to improve functional and aesthetic outcomes in full-thickness wound repair.

Methods: A 3D skin substitute containing iNeurons, human dermal microvascular endothelial cells (HDMECs), fibroblasts, and hair follicle spheroids was developed. Human dermal fibroblasts were directly transdifferentiated into induced glutamatergic neurons (iNeurons) using a defined small-molecule cocktail over 18 days. HDMECs, fibroblasts, and keratinocytes were isolated and cultured from human foreskin. Hair follicle spheroids were prepared from dermal papilla cells and keratinocytes and integrated into the same construct.

Results: In 3D skin substitutes, HDMECs formed interconnected blood and lymphatic capillary networks. iNeurons maintained their phenotype and density within the construct and enhanced blood capillaries, but not lymphatic formation. iNeurons exhibited mature neuronal morphology and expressed key markers, including PGP9.5, VGLUT1, and TUBB3. Notably, blood capillaries were also found in close proximity to the hair follicle spheroids, suggesting possible functional interplay.

Conclusions: We successfully engineered a human skin substitute integrating vascular, neural, and follicular elements. This biomimetic construct recapitulates native skin architecture, supporting reinnervation and hair follicle integration. Our approach offers a promising strategy for next-generation skin grafts that improve structural, sensory, and cosmetic outcomes—using only a small skin biopsy from the patient.

O4. ADJUSTING THE HANDLING OF A CELL FREE HYDROGEL SYSTEM BASED ON WHARTON'S JELLY EXTRACELLULAR MATRIX TO OPTIMIZE FACIAL NERVE REGENERATION

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Background: Nerve regeneration is a complex process involving axonal sprouting, myelination, and the re-establishment of functional neuronal circuits. Traumatic nerve injury triggers a local inflammatory response, which plays a critical role in effective regeneration. In this study, we aimed to enhance nerve repair across long gaps by modulating the early immune response.

Methods/Results: We developed a novel porous, freeze-dried Wharton's jelly (WJ) hydrogel incorporating a tannic acid (TA) reservoir within a nerve filler conduit. Rheological and compression testing showed a marked increase in storage and elastic moduli compared to TA-free hydrogels, reaching 1.91 ± 0.08 kPa and 1.38 ± 0.01 kPa, respectively. The TA-WJ hydrogel displayed a swelling ratio of 679 ± 221% and a contact angle of 50 ± 8°. Biodegradation assays revealed over 30% of the material remained after 48 hours of collagenase treatment. Structural analysis revealed a conduit with a micro-grooved luminal surface and a porous, multi-channelled wall, with a thickness below 0.8 mm meeting nerve conduit design guidelines. *In vitro* studies with neutrophils and monocytes, as well as *in vivo* subcutaneous implantation, demonstrated anti-inflammatory properties that may promote a favourable immune environment. In a rat model of buccal branch facial nerve injury, TA-WJ conduits supported functional recovery, as evidenced by improved clinical scores compared to silicone controls. Histological analysis at eight weeks post-implantation confirmed nerve thread formation and neovascularization around the regenerating nerve.

Conclusions: Altogether, these results position the TA-WJ hydrogel conduit as a promising and multifunctional platform for peripheral nerve regeneration, combining structural support, immunomodulation, and pro-regenerative properties.

O5. GENERATION OF A STEM CELL-DERIVED CELL THERAPY PRODUCT FOR HUNTINGTON'S DISEASE

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Background: Huntington's disease (HD) is a progressive, incurable neurological disorder characterised by motor, cognitive and psychiatric features. Early atrophy within a subcortical region of the brain, particularly the medium spiny neurons of the striatum (MSNs), represents a key neuropathological feature of HD. Proof-of-concept that cell replacement therapy can alleviate or halt progression of some motor and cognitive features of the disease has been established using foetal-derived MSNs in both preclinical rodent models and in early clinical trials. Stem cell-derived MSNs are required for widespread clinical application and, to date, some *in vitro* differentiation strategies have been established that are capable of generating high proportions of MSN progenitors. To develop a stem cell-derived MSN progenitor cell product to repair the neural circuitry affected by HD.

Methods/Results: Here, we report the generation of a novel human stem cell-derived cell therapy product (RHD001). This product is generated using a modification to the Activin-A differentiation protocol and it has been optimised for *in vivo* transplantation into the brain. We have conducted single-cell RNAseq on our RHD001 progenitor cells to confirm expression of key markers of authentic MSN progenitor cells at the point of cryopreservation. Post-transplantation, we have confirmed survival, integration and expression of key MSN markers by immunocytochemistry. Using the monosynaptic tracing technology, we have demonstrated host-to-graft synapse formation. Slice electrophysiology and behavioural assays (apomorphine-induced drug rotation, cognitive testing using the lateralised reaction time task) establish the functional efficacy of the RHD001 cell therapy product *in vivo*.

Conclusions: Going forward, we aim to complete safe and toxicity testing, in order to commence a first-in-person clinical trial in people affected by HD.

O6. CORNEAL EPITHELIAL WOUND HEALING BY PURIFIED COMPONENTS FROM IMMORTALIZED STEM CELLS FROM HUMAN EXFOLIATED DECIDUOUS TEETH

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Background: Stem cell-based regenerative medicine has been studied. One clinical therapy is mesenchymal stem cells (MSC) transplantation into patients with graft-versus-host disease (GVHD). However, the transplanted cells are immediately destroyed due to immune reactions, which reduces the benefits of stem cell transplantation. Therefore, alternative therapies are more realistic for clinical use. In this study, we aimed to take advantage of the therapeutic efficacy of MSCs using multiple cytokines released by stem cells from human exfoliated deciduous teeth (SHEDs), and studied the efficacy for treating ocular surface diseases.

Methods: We purified components from the conditioned media of immortalized SHEDs (IM-SHED-CM) and evaluated intracellular dehydrogenase activity, cell migration and antioxidant stress using some cells including human corneal epithelial cells. We administered IM-SHED-CM, and the purified component as eyedrops to a chronic GVHD mouse model with sever corneal epithelial damages and examined the efficacy from corneal analyses.

Results: The purified component of > 3.5 kD and 50-100 kD from IM-SHED-CM had higher efficacy than the original IM-SHED-CM in terms of intracellular dehydrogenase and cell migration in which intracellular signal transduction was activated via receptor tyrosine kinases, and the glutathione peroxidase and reductase system was highly active. In vitro assay demonstrated that the purified component significantly enhanced human corneal epithelial wound healing. Furthermore, from in vivo assay, the purified component-based eye drops reduced corneal epithelial damage, inflammatory cell infiltration, and oxidative stress in the corneal epithelium and maintained the expression of limbal stem cell markers in the cGVHD mouse model, indicating the earlier recovery with the eye drops.

Conclusions: IM-SHED-CM and the purified components enriched with factors that promote epithelial wound healing. The eye drops could be a novel treatment for corneal epithelial damage, emphasizing the importance of bioactive factors in SHED-CM for wound healing and immune.

O7. DEVELOPMENT OF EXTRACELLULAR MATRIX PROTEIN-DECORATED HYDROGELS FOR VASCULARISATION

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Background: The development of vasculature to sustain 3D tissues remains a major challenge. Extracellular matrix (ECM) properties like adhesion ligand presence and growth factor binding critically influence cell behaviour, including network formation by vascular cells. Hyaluronic acid (HyA) hydrogels offer potential as tuneable, biocompatible synthetic ECMs (sECMs) to control cell behaviour. We hypothesise that decoration of HyA-based sECMs with ECM proteins native to the vascular microenvironment can promote 3D vascular network formation by encapsulated human vascular cells.

Methods: HyA was modified by addition of acrylate groups (AcHyA) and modification confirmed by Nuclear magnetic resonance (NMR). Fibronectin (FN) or laminin (LM) was conjugated to AcHyA via sulfide-mediated Michael addition, and HyA sECMs formed using dithiol crosslinkers. HyA sECM swelling, degradation, and storage modulus were evaluated by standard methods. Vascular endothelial growth factor (VEGF) sequestration and diffusion were assessed by ELISA and fluorescence recovery after photobleaching (FRAP). Vascular cells were differentiated from human induced pluripotent stem cells (hiPSCs), encapsulated in ECM protein-decorated HyA hydrogels, and visualized with F-actin staining.

Results: NMR confirmed ~22% acrylation of HyA and conjugation of FN or LM. ECM protein conjugation did not alter swelling, degradation or storage modulus (~600-800Pa) of HyA sECMs. ELISA demonstrated significantly enhanced VEGF retention in ECM protein-decorated sECMs compared to AcHyA-only (~55% compared to ~65% over 5 days), supported by FRAP demonstrating significantly reduced VEGF diffusion in FN and LM-decorated HyA sECMs. Vascular cells encapsulated in FN or LM-decorated HyA sECMs exhibited significantly greater elongation over 7 days, which was confirmed by increased cell area and reduced cell circularity.

Conclusions: Decoration of AcHyA sECMs with FN or LM, ECM proteins native to the vascular microenvironment, improved VEGF sequestration and vascular cell elongation, indicative of early angiogenesis, without altering HyA sECM biophysical properties. This system offers promise as a tuneable sECM platform to recapitulate and study 3D vascularization.

O8. IRON-INDUCED CHANGES IN DERMAL FAT-FIBROBLAST-SIGNALLING AXIS DRIVE DISEASE PROGRESSION IN LIPODERMATOSCLEROSIS

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Background: In this study, to prevent lipodermatosclerosis, a skin disease still understudied, we investigate the pathological mechanisms underlying the development and progression.

Methods/Results: We generated a mouse model that mimicked skin changes observed in patients with lipodermatosclerosis, including an iron-overloaded dermal and adipose layer, fat loss and immune cell infiltrates. Our analyses of the iron-overloaded skin layer indicate iron-related changes in the dermal fat-fibroblast signalling axis that contribute to disease-related alterations in fibroblast functions. Single-cell RNA sequencing analyses revealed two fibroblast clusters, progenitor-like Col3+ Pi16+ Fib1 and metabolically active Fabp4+ Cd36+ Fib2, which showed significant changes and a proportional shift in the iron-treated skin. Upregulated pathways in Fib1 are associated with response to metal ions, Foxo signalling and apoptosis, suggesting that their loss is due to iron-induced stress and cell death. In contrast, Fib2 enrichment is due to increased proliferation, which could be mimicked by the addition of iron. Meaning that excessive iron even promotes the expansion of Fib2. Furthermore, differentiation trajectory analyses (Pseudotime and CytoTrace) revealed that cells in Fib2 are less differentiated in the iron-treated skin. This might be linked to downregulated Wnt signalling in the fibroblast cluster that was confirmed by reduced Wnt gene expression and β-catenin staining in the skin tissue. Interestingly, Fib2 in the iron-treated skin presents up-regulated pathways associated with lipid transport and fatty acid oxidation, which might be related to alterations in the dermal white adipose tissue (dWAT) layer. The thickness of the dWAT in the iron-treated skin is reduced due to adipocytes undergoing lipolysis, and an additional reduction of adipocyte stem cells in the dWAT of iron-treated mice. In fact, *in vitro* experiments on adipocyte-derived MSCs confirmed that high iron levels induce lipolysis while preventing adipogenesis.

Conclusions: The combination of both events could eventually lead to complete fat loss and disease progression.

O9. IDENTIFICATION AND CHARACTERIZATION OF AKR101 AS POTENTIAL NEW THERAPEUTIC FOR CHRONIC WOUNDS

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Background: Wound exudates (WEs) contain the pathogenic drivers of chronicity of individual wounds. They can transfer the clinical phenotype to primary human dermal fibroblasts in cell culture, which we exploited to establish a cell-based wound assay system, and to screen substance libraries for new compounds with potential for chronic wound therapy.

Methods: In miniaturized cell assays (384-well format), WEs from individual patients and test compounds or controls were simultaneously applied to primary human dermal fibroblasts. Proliferation (after 72 hours) and fibroblast-derived matrix formation (after 8 days) were quantified by protein staining of fixed cells. Production of extracellular matrix (ECM) proteins and inflammatory cytokines was assessed by qRT-PCR and/or ELISA.

Results: Using our cell-based wound assays, we identified substance classes that could counteract the negative effects of chronic WEs in cell culture. Extended characterization of selected compounds revealed the low molecular weight compound AKR101 as the most promising drug candidate. In the presence of inhibitory WEs, AKR101 restored fibroblast proliferation and enhanced 3-dimensional matrix formation, accompanied by normalized expression of the ECM components collagen 1 and collagen 3. Moreover, it dose-dependently inhibited the production of the inflammatory cytokine IL-1ß. These *ex vivo* parameters represent changes that are indicative of a transition from a chronic/non-healing to a healing phenotype. The extent of improvements was patient-specific but wound etiology-independent. Rescue effects of AKR101 were observed with ~90% of the 101 non-healing WEs tested, which is superior to the marketed wound therapeutics platelet-derived growth factor and silver sulfadiazine.

Conclusions: Compound AKR101 was shown to reverse the detrimental effects of exudates from a multitude of chronic wound patients. It was therefore identified as a potential new therapeutic to promote wound healing. AKR101 is currently being developed for a clinical proof-of-concept study in chronic venous leg ulcer patients.

O10. A CLINICAL TRIAL EXAMINING FICUS SEPTICA LATEX FOR THE TREATMENT OF SMALL CUTANEOUS ULCERS IN PAPUA NEW GUINEA

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Background: In Papua New Guinea (PNG), cutaneous ulcer disease (CUD) affects millions of children, but is generally left untreated due to a lack of access to basic medicines. Metagenomics studies reveal *Haemophilus ducreyi* and *Streptococcus pyogenes* as key pathogens associated with CUD in PNG. This suggests that CUD could be treated with topical antiseptics, including traditional antibacterial plant saps, which are readily available even in remote areas that lack basic primary healthcare.

Methods: Ethnobotanical surveys were used to identify medicinal plant saps with a history of use in treating CUD. Activity-guided fractionation and NMR were used to identify antibacterial plant metabolites. Antibacterial mechanisms were examined using bacterial cytological profiling. Pro-inflammatory responses of M1 macrophages and neutrophils were measured by ELISA. A cluster-randomised clinical trial, assessing *F. septica* versus Savlon cream was carried out in PNG. Fifty participants were enrolled per treatment arm, each presenting with cutaneous ulcers less than 1cm in diameter. Assessment of healing was carried out by a panel of dermatologists examining blinded photographic evidence of ulcer healing at day 7 and 14 compared to baseline.

Results: The latex of *F. septica* contains the antibacterial alkaloid ficuseptine which showed activity against relevant pathogens *in vitro*. Bacterial cytological profiling of ficuseptine suggests an antibacterial mechanism of action targeting cell wall synthesis, a mechanism rarely reported for plant secondary metabolites. Furthermore, the latex was found to modulate the pro-inflammatory responses of M1 macrophages and neutrophils suggesting possible effects on innate immune responses in the ulcers. In the clinical trial, at day 14, *F. septica* latex was found to be non-inferior to Savlon cream providing similar levels of healing.

Conclusions: *F. septica* latex presents multiple mechanisms of action and is a potential treatment option for communities living in remote areas of PNG that lack access to basic healthcare.

O11. A NOVEL POPULATION HEALTH-BASED PIPELINE TO SCREEN FOR SCARRING GENES REVEALS A ROLE FOR LGR4 IN SKIN REPAIR

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Background: Skin wound repair is a complex process that requires the orchestrated response of different cell types to repair the damaged tissue and restore function. Small skin wounds heal within days, but larger wounds generally heal leaving collagenous scars, which can impact tissue function. Given that human populations exhibit a wide variation in scarring phenotypes, we reasoned that a genetic association approach might provide an alternative way to reveal genes associated with scarring.

Methods: We used Bacillus Calmette-Guérin (BCG) vaccination scars as a natural experiment where patients received standardised “wounds”. We measured BCG scars of 1011 participants from the Bristol-based Avon Longitudinal Study of Parents and Children (ALSPAC) cohort and performed GWAS on scar sizes. Next, we used zebrafish to validate our results by generating mutant zebrafish encoding a truncated Lgr4 variant and combining them with *in vivo* imaging of fluorescent transgenic reporters to characterise wound re-epithelialisation, angiogenesis, inflammatory response, and collagen deposition.

Results: Here we identified an intronic SNP associated with decreased scar size in the *LGR4* gene. *LGR4* encodes a G protein-coupled receptor involved in WNT signalling. Wounds of mutant *lgr4* larvae show decreased macrophages, fewer classically activated (M1) macrophages, and increased cell proliferation. *Lgr4* mutants also exhibit decreased collagen fibre alignment in the wound.

Conclusions: Our findings set a precedent for the use of human population data to identify new loci involved in scarring in an unbiased way. Our validatory experiments on animal models test the function of these genes in wound healing and scarring, with the long-term goal of developing anti-scarring therapeutics. We have now begun interrogating other larger cohorts in the UK and overseas where other skin wounding interventions and general fibrosis conditions have been measured.

O12. CCN3-BASED PEPTIDE BLR-200 HAS ANTI-FIBROTIC PROPERTIES IN SYSTEMIC SCLEROSIS

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Background: Scleroderma is an autoimmune connective tissue disease characterized by fibrosis of the skin and internal organs. We have shown that this fibrosis is induced/maintained by an autocrine proadhesive signalling loop mediated by YAP and the YAP target CCN2. The CCN family of matricellular proteins have subtle pro-adhesive effects *in vitro*. Collagen-lineage (universal) fibroblast-specific expression of CCN2 is required for fibrosis in variety of *in vivo* models. Anti-CCN2 therapies have failed clinically, likely due to functional redundancy in the CCN family. Conversely, the CCN family member CCN3 is inherently antifibrotic. We have identified a CCN3-based peptide that mimics the antifibrotic action of CCN3. We are developing this peptide, BLR-200, as our lead drug. If BLR-200 has antifibrotic properties in models of scleroderma, and its mechanism of action is unclear.

Methods: An adhesion assay screen was used to initially identify BLR-200. We used the bleomycin-induced model of scleroderma, lineage tracing, spatial transcriptomics, real-time polymerase chain reaction (RT-PCR), proteomic and RNAseq/scRNAseq analyses to evaluate the anti-fibrotic properties of BLR-200.

Results: *In vitro*, BLR-200 impairs, but does not block adhesion of primary human foreskin fibroblasts to type I collagen. *In vivo*, BLR-200 blocks bleomycin-induced increases in skin thickness, matrix production and myofibroblast differentiation ($N=8$, $p<0.05$). Trajectory and scRNAseq analyses show that BLR-200 acts by impairing the activation of the pro-fibrotic engrailed/collagen8A1-positive subset of collagen-lineage universal fibroblasts and promoting the induction of a pro-regenerative engrailed/sfrp2-positive subset. This activity is accompanied by reduced expression of focal adhesion, wnt, oxidative phosphorylation and matrix-associated clusters, and alpha-smooth muscle actin, Smad3, YAP1, PLOD2, tenascin-C, CCN1 and CCN2 mRNA expression ($N=6$, $p<0.05$).

Conclusions: BLR-200 is the only drug in development that targets both CCN1 and CCN2. BLR-200 could be a novel treatment to block scleroderma skin fibrosis.

O13. DEVELOPMENT OF A FLIGHTLESS I NEUTRALIZING ANTIBODY THERAPY FOR THE TREATMENT OF BURNS

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Background: The actin remodelling protein, Flightless I (Flii), is present at high levels in damaged tissues including burns, fibrotic lesions and scars. We have generated mouse monoclonal antibodies capable of binding Flii and neutralizing its effect (FnAb), effective in promoting the healing of burns when injected into the edges of mouse burn injuries. Given that local cutaneous injection of FnAb to large surface area burns is sub-optimal, we aimed to develop an alternative method to deliver these healing-promoting antibodies in a clinically relevant format.

Methods: Using a murine model of partial thickness burn injury (n=8), we investigated if systemic injection or a topical hydrogel formulation of FnAbs could improve burn outcomes. IVIS imaging was used to visualize labelled FnAb following injection by IP route. Wound closure was determined macroscopically, with immunohistological analysis performed to determine the effect on inflammation and angiogenesis within the burn site.

Results: Labelled FnAb was observed to localize within the burns up to 7 days. Burn assessment, post IP injection of FnAb, showed no overall change in rate of wound closure but significantly reduced inflammation and improved angiogenesis. The FnAbs were subsequently loaded into a topical hydrogel formulation and compared with industry standard burn gel, Solosite. We found that application of the FnAb hydrogel improved the rate of healing of partial thickness scald burns particularly in female mice, with wounds up to 50% smaller on day 10.

Conclusions: These results suggest that systemic delivery of FnAb is a promising approach for the treatment of burns with improved angiogenesis and reduced inflammation observed and direct topical application of FnAb-loaded hydrogel provides faster rate of wound closure. Overall, these studies suggest that a combination of topical FnAb hydrogel formulation with a systemic treatment may be a promising approach for improving burn injury repair.

O14. TOPICAL EXOSOMES MEDIATE WOUND HEALING BENEFIT VIA MODULATION OF INNATE IMMUNE CELLULAR MILIEU

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Background: Chronic ulcers are a widespread complication of diabetes, with current treatments failing to address the underlying pathology. Multipotent stromal cell (MSC) exosomes offer a promising tissue engineering approach to enhance wound healing. Here, we evaluate efficacy of exosome treatment and molecular/cellular consequences in mouse diabetic wounds.

Methods: We utilized a full-thickness stented skin wound in $LepR^{db/db}$ diabetic mice, that exhibit severely delayed closure. We isolated exosomes from human bone-marrow MSC supernatant via differential ultracentrifugation. At post-operative day 1 (POD1), we administered a single topical dose of $1e^9$ exosomes or PBS. We monitored wounds to closure and sampled tissues at intermediate timepoints. We performed multiplexed immunostaining and single cell RNA analysis. We administered clodronate liposomes to deplete macrophages in diabetic mice.

Results: Exosome-treated diabetic wounds showed 88.9% reduction in pathological time to closure, compared to control ($p<0.01$), and on par with non-diabetic wild type (WT) ones. By POD10, the wound epithelial gaps were similar between WT and exosome-treated diabetic ones at 4.95 ± 0.95mm and 5.60 ± 1.60 mm, respectively. Exosome-treated diabetic wounds develop extensive $CD31^+$-neovascularization in αSMA^+-granulation tissue, with exosomes in endothelial cell cytoplasm, in proximity to $F4/80^+$macrophages. This is in stark contrast to control diabetic wounds with minimal granulation tissue. scRNASeq revealed increased numbers of monocyte/macrophages in exosome-treated diabetic wounds, compared to control ones, as well as upregulation of genes that regulate interactions with vascular and lymphatic endothelial cells. Myeloid leukocyte migration was among biological processes regulated by exosome treatment. The wound-healing benefit was negated in macrophage-depleted diabetic wounds, underscoring the critical role of macrophages in exosome-induced healing.

Conclusions: Our study provides mechanistic insight behind the therapeutic efficacy of exosome treatment, and offers an approach to study endogenous temporally regulated mechanisms of macrophage plasticity and functions through the progressive wound healing phases.

O15. TARGETING PTP1B ENHANCES DIABETIC WOUND HEALING BY MODULATING MACROPHAGE POLARIZATION VIA HO-1 PATHWAY

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Background: Diabetic foot ulcers (DFUs) are a major complication of diabetes mellitus, characterized by persistent inflammation, impaired angiogenesis, and oxidative stress. Protein tyrosine phosphatase1B (PTP1B), a negative regulator of insulin signaling, is overexpressed in diabetic condition. This study aimed to investigate the therapeutic potential of PTP1B inhibition in diabetic wound healing and to elucidate the underlying mechanisms.

Methods: Diabetes was induced in male C57BL/6 mice via intraperitoneal streptozotocin (STZ) injections (50 mg/kg) for 5 consecutive days. After 6 weeks, two 6 mm full-thickness excision wounds were made on the dorsum of each mouse. Wounds were treated topically twice daily until day 3 with either vehicle (control), Trodusquemine (PTP1B inhibitor), Protoporphyrin-IX-zinc(II) (heme oxygenase1 inhibitor, HO1i), or PTP1Bi+HO1i. Wound healing was monitored for 10 days. Inflammatory macrophage profiles (M1/M2), angiogenesis (CD31), proliferation (Ki67), HO-1 expression/activity, and ROS levels were assessed. *In vitro*, THP-1 cells were differentiated into macrophages, treated with PTP1Bi, and stimulated with LPS for 24 hours under high glucose or normoglycemic conditions. Polarization, cytokine expression, ROS production, and HO-1 levels were evaluated. PTP1B expression was elevated in diabetic skin. PTP1B inhibition significantly improved wound closure (day 10: 1.1±0.2% vs 7.2±1.4% in control, $p<0.01$), reduced M1/M2 ratio, increased vascular density (147.4±5.5%) and proliferation (141.1±5.9%), and decreased ROS levels (68.1±6.1%). HO-1 expression and activity were strongly upregulated (219.4±20.7% and 170.4±15.7%, respectively), and HO-1 inhibition reversed the beneficial effects. *In vitro*, PTP1B inhibition promoted anti-inflammatory M2 polarization, suppressed IL-6, IL-1β, MCP-1, and elevated HO1 expression.

Conclusions: These findings demonstrate that PTP1B inhibition enhances diabetic wound healing, angiogenesis and proliferation by modulating the inflammatory and oxidative microenvironment through HO-1. PTP1B represents a promising therapeutic target for the treatment of chronic DFUs.

O16. SOLUBLE CD83 ENHANCES INTESTINAL WOUND HEALING IN DSS-INDUCED COLITIS

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Background: The immune modulatory molecule soluble CD83 (sCD83) has recently emerged as a potential mediator of tissue regeneration. Here, we investigate its role in intestinal wound healing using a dextran sulfate sodium (DSS)-induced colitis model, which is characterized by epithelial injury and inflammation of the gut mucosa.

Methods: Mice treated with sCD83 during the acute phase of colitis displayed markedly improved clinical outcomes, including reduced weight loss and enhanced mucosal healing, as assessed by colonoscopy and validated by histological scoring of hematoxylin and eosin (H&E)-stained colonic sections.

Results: sCD83-treated animals demonstrated significantly better preservation of epithelial architecture and reduced ulceration compared to vehicle-treated controls. Analysis of colonic lamina propria immune cells via flow cytometry revealed an increased infiltration of macrophages and eosinophils, alongside a reduction in neutrophils. These data suggest that sCD83 may promote a wound-healing immune phenotype and support the hypothesis that sCD83 fosters a pro-reparative microenvironment.

Conclusions: In summary, our results identify sCD83 as a promising candidate for enhancing intestinal wound healing. These findings open new avenues for therapeutic modulation of mucosal repair in inflammatory bowel disease and other conditions marked by impaired intestinal regeneration.

ETRS YOUNG INVESTIGATOR AWARD ORAL PRESENTATION ABSTRACTS

Y1. UNDERSTANDING CONFINED FUNCTIONS OF NEUTROPHILS IN THE PATHOPHYSIOLOGY OF DIABETIC WOUND HEALING

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Background: Neutrophils are among the first immune cells recruited to injury sites, where they combat infection through phagocytosis, degranulation, and formation of neutrophil extracellular traps (NETs). They also release cytokines and growth factors to promote inflammation and tissue repair. However, sustained neutrophil activation is linked to chronic inflammation and impaired healing, as seen in diabetic ulcers. The specific alterations in neutrophil function in diabetic wound healing remain poorly understood.

Methods/Results: We studied the spatiotemporal organization of neutrophils in full-thickness wounds in wildtype mice. Neutrophils infiltrated from the wound bed and accumulated in the eschar. In diabetic mice, infiltration was delayed and prolonged, with neutrophils remaining in deeper tissue layers where they formed NETs. Similarly, human diabetic wounds showed more neutrophils and NETs in the wound bed, whereas in acute wounds they localized mainly to the eschar. To investigate underlying mechanisms, we analysed peripheral blood neutrophils from healthy and diabetic donors using multicolour flow cytometry. Diabetic neutrophils showed altered surface marker expression, including reduced levels of CD10 (maturation), CD47, and CD13 (migration). We also examined low-density neutrophils (LDNs), known to increase in chronic inflammatory states. Diabetic donors exhibited a higher frequency of LDNs. Moreover, these donors showed an increased prevalence of a CD16 low LDN subset, which displayed an exhausted phenotype characterized by downregulated chemokine receptors and upregulated degranulation markers. Additionally, we demonstrated that LDNs express platelet-associated surface markers,

Conclusions: Given that platelets are often pre-activated in diabetes, we hypothesize a link between platelet activity and LDNs. Co-culture of neutrophils with platelet releasates reproduced the LDN marker profile, supporting this connection**.** Future studies will examine diabetic platelet releasates' effects on neutrophils. Our findings suggest that altered surface markers in diabetic neutrophils contribute to their tissue retention. The rise of platelet-associated LDNs in diabetes may further impair early wound responses, disrupting healing and promoting chronic inflammation.

Y2. SYNERGISM OF ENDOTHELIAL CELL DEATH AND FIBROBLAST SENESCENCE IN DIABETIC ANGIOPATHY

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Background: Global incidence of obesity and type 2 diabetes (T2DM) is increasing, placing significant clinical and socioeconomic burden on society. Non-healing wounds constitute a serious complication of obesity/T2DM and are mainly attributed to impaired angiogenesis. Illuminating the underlying pathogenesis of dysregulated angiogenesis may help to identify new therapeutic targets. This study explored the impact of saturated and mono-saturated fatty acids – elevated in both obesity/T2DM - on angiogenesis and its disruption.

Methods: We implemented an *in vitro* tube formation assay that relies on the interaction between endothelial cells and fibroblasts. Fatty acids, representative of those found to be increased in obesity/T2DM, significantly impaired angiogenesis in a concentration-dependent manner. Subsequent studies were performed to determine the underlying causes of this impairment and to explore the endothelial-fibroblast cross-talk.

Results: Fatty acids induced endothelial cell death through ferroptosis and caspase-3 dependent apoptosis. Fatty acids markedly upregulated the expression of pro-ferroptotic factors, increased intracellular free iron, reactive oxygen species and lipid peroxidation products in endothelial cells. Concurrently, endothelial cells exhibited a reduction of GPX4 levels and lower GSH/GSSG ratio indicative of their disturbed antioxidant defence. In contrast, fibroblasts exposed to fatty acids exhibited higher expressions of p21, p27 indicative of cellular senescence, along with higher senescence-associated beta-galactosidase activity. Senescent fibroblasts also showed reduced Ki67, reflecting reduced cell proliferation. Consistent with observations in human diabetic wounds, full thickness wounds in diabetic (*db/db*) mice exhibited reduced vascularization. In addition, reduced endothelial GPX4 expression and higher p27, p21 and lower Ki67 expression in fibroblasts were detected in the skin of *db/db* mice.

Conclusions: Our findings suggest that T2DM/fatty acids concomitantly induce endothelial death and fibroblast senescence which suppress proliferation, migration, sprouting of endothelial cells and, hence, new vessel formation. These findings advance our understanding and hold promise for the development of new therapeutic strategies of difficult-to-heal diabetic wounds.

Y3. IDENTIFYING NOVEL WOUND EXTRAVASATION GENES USING A DROSOPHILA-TO-MURINE PIPELINE

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Background: Inflammation is an ancient and pivotal response that has evolved to respond to any tissue wound or infection. Leukocyte extravasation from circulation is an early, rate-limiting step of most inflammatory responses and is a highly complex, polygenic and regulated trait, involving the interaction of multiple molecules expressed by both immune and vasculature tissues. For decades studies of extravasation have relied on *in vitro* and small mammalian models, with research being long-winded and expensive. To overcome this issue, we have developed a multi-model pipeline, with the initial screening and characterisation of novel extravasation genes performed in *Drosophila* whose mammalian orthologues are then followed-up in mouse models.

Methods/Results: Extravasation involves the vasculature and immune cells working together and thus a subtractive screening approach was required to delineate the autonomy of gene function during diapedesis. Our first *Drosophila* immune cell “hit”, the G-protein couple receptor Tre1/Gpr84, has now been successfully validated in a mouse model and identified as critical for proper and timely neutrophil detachment from the pericyte layer in several murine models of inflammation. In *Drosophila*, we have shown that Tre1 acts to regulate the cytoskeleton and integrin focal adhesion points via active RhoA localisation to the lagging tail of extravasating cells. Tre1 and Gpr84’s mechanism appears to be conserved between fly and mouse which exemplifies and validates this multi-species screening approach.

Conclusions: The further screening of genes and potential pathways discovered with the fly will have significant clinical relevance as immune cell extravasation underpins many physiological and pathological responses associated with inflammation gone awry.

Y4. PROTEOMIC PROFILING OF PLATELET-RICH PLASMA TO PREDICT THERAPY OUTCOMES IN COMPLEX WOUNDS

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Background: Complex wounds are an increasing challenge in regenerative medicine, driven by global aging. Among the biological therapies, autologous platelet-rich plasma (PRP) holds promise in enhancing wound healing. However, the clinical application of PRP is limited by the absences of standardized protocols and a lack of information on variables that could help personalize the therapy. This study aims to identify PRP protein biomarkers linked to healing efficacy, guiding personalized therapies.

Method: A prospective observational study was conducted on thirty-two patients with chronic wounds treated with PRP, recruited from an intermediate care hospital. PRP was obtained by sequential blood sample centrifugation (400g for 9 min, then 900g for 10 min). Platelets were activated with calcium gluconate (10%) in the presence of heparin (2 IU/ml) at 37ºC for 30 min, then centrifuged at 5000g for 15 min to remove residual platelets. Following, the proteomic processing comprised abundant protein immunodepletion, peptide digestion, and LC-MS/MS profiling. Data analysis was performed using bioinformatics tools.

Results: We identified a total of 849 proteins, of which 154 were classified as differentially expressed proteins (DEPs) in at least one treatment outcome (healed, stagnant, or complicated) contrast. Hierarchical clustering of DEPs grouped patients based on their treatment outcomes. Sparse Partial Least Squares-Discriminant Analysis (sPLS-DA) demonstrated a clear distinction between patient groups according to their treatment outcomes based on the abundance of their total proteins. The combined analysis of DEPs and sPLS-DA identified a subset of proteins with potential prognostic value for treatment outcome.

Conclusions: Proteomic profiling of PRP samples from patients with complex wounds treated with PRP revealed significant correlations with treatment outcomes. The identified protein biomarkers related to these outcomes provide essential diagnostic and prognostic information, which is crucial for optimizing the personalization of PRP therapy. Larger patient studies are needed to validate the clinical applicability of these results.

Y5. ALGINATE-BASED HYDROGELS LOADED WITH HUMAN BETA-DEFENSIN-2 ENHANCE HEALING OF INFECTED AND NON-INFECTED WOUNDS IN A DIABETIC MOUSE MODEL

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Background: Diabetic foot infections (DFIs) are among the most serious and common complications in patients with diabetes, often caused by persistent microbial colonization of non-healing wounds. As antimicrobial resistance continues to rise, there is an urgent need for novel therapeutic approaches. In this study, we explore the combination of the dual antimicrobial and pro-healing properties of antimicrobial peptides (AMPs) with the intrinsic characteristics of the alginate polymer as an encouraging multifunctional strategy to address the challenges of DFIs.

Methods: Using ionic crosslinking with calcium sulphate, we designed alginate-based hydrogels with a nanometric porous structure capable of sustained delivery of the AMP human β-defensin-2 (hBD-2) over time for promoting wound healing. The effects of these hydrogels were assessed in streptozotocin-induced diabetic mouse models with full-thickness excisional wounds, either with or without methicillin-resistant *Staphylococcus aureus* (MRSA) infection.

Results: In the non-infected model, hBD-2 hydrogels significantly accelerated wound closure and improved wound maturation, by stimulating re-epithelialization and tissue remodelling. Moreover, hBD-2 hydrogels decreased the wound overall microbial load, the M1/M2 macrophage ratio, and the production of reactive oxygen species, while increasing cell proliferation, neovascularization, and COL1A1 deposition. In the MRSA-infected model, hBD-2 hydrogels also improved wound closure, yet to a lesser extent than in the non-infected model. Furthermore, hBD-2 hydrogels attenuated the wound MRSA load, while decreasing the inflammatory state. Finally, hBD-2 hydrogels slightly improved cell proliferation and neovascularization, ultimately supporting the evidence of an early progression of tissue regeneration, despite the hostile infected environment.

Conclusions: Overall, these findings underline the potential of hBD-2 hydrogels as a promising multifunctional therapeutic approach for chronic wounds, by offering combined antimicrobial, anti-inflammatory, and regenerative benefits, even under challenging MRSA-infected wound conditions.

Y6. THE IMMUNEMODULATOR SOLUBLE CD83 AMELIORATES WOUND HEALING QUALITY IN A MURINE PRESSURE ULCER MODE BY INDUCING TISSUE REPAIR MACROPHAGES

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Background: Pressure Ulcers (PUs) represent a major challenge for clinicians and healthcare personnel, as they require special, costly and time-consuming care strategies. Particularly elderly patients suffer from PUs, as wound healing deficiencies prevail with age. Therapy is limited to symptomatic treatment and nursing experience, highlighting the need for novel therapeutic options. One promising candidate, the immunemodulatory soluble CD83 (sCD83) molecule has shown pro-regenerative capacities for the treatment of acute wounds. To assess its efficacy in the context of hard-to-heal conditions, we induced ischemia–reperfusion (I/R) wounds, emulating the pathogenesis of pressure ulcers.

Methods/Results: Mice were subjected to two cycles of 12 mm magnets applied on the dorsal skin for 12 hours, followed by 12 hours of recovery. Systemic treatment commenced on day 5 with either sCD83 (100 µg/mouse) or mock, monitoring the wound closure. Obtained wound biopsies were analysed by qPCR, H&E staining or flow cytometry. Noteworthy, histological analyses show significantly improved skin architecture on day 18. On cellular level, flow cytometric analysis revealed lower levels of neutrophils, contrasting increased levels of T-cells upon sCD83-treatment, indicating an accelerated progression throughout the wound healing process. Analyses of day 8 wound biopsy transcriptome profiles, unravelled an early boost of the inflammatory phase, as indicated by higher RNA-levels of pro-inflammatory mediators, such as *Tnfa* and *Cxcl10*. Concomitantly, sCD83 promotes the expression of *Il10*, *Ym1*, and *Tgfb1*, prominent markers of tissue-repair macrophage polarization, which was reflected by an increased prevalence of these cells sampled wound biopsies. Notably, tissue-repair macrophages are crucial to control inflammation and attract fibroblasts, guiding the wound healing process into the pro-regenerative phases and coordinating tissue repair.

Conclusions: Our findings expand the manifold capacities of sCD83's for the treatment of acute and PUs, paving the way for future clinical applications to address this urgent medical need.

POSTER PRESENTATION ABSTRACTS

P1. NEW HYBRID ORGANO-INORGANIC MATERIALS BASED ON [BIOACTIVE MOLECULE@POLYSACCHARIDE@SILICA] COMPOSITES: PREPARATION AND PROPERTIES

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Background: Modification of the silica surface with polysaccharides with different types of functional groups (chitosan, dextran, etc.) is a way to create new organic-inorganic hybrid materials [1, 2] that new sorption characteristics will distinguish, defined both by the functional groups of the polysaccharide and by the structure of the adsorption layer with free “loops” and “tails”, which is determined by the calculated values of the parameter ***p***. To develop three-component systems [bioactive molecule@polysaccharide@silica/ceria], studies of the adsorption-desorption processes and thermal transformations on the surface of the original and modified oxides of a number of biologically active compounds were carried out: (i) anesthetics - lidocaine, novocaine; (ii) antiseptic - supramolecular complex iodine-chitosan; (iii) angioprotector – venoruton; (iv) antioxidants – p-hydroxy cinnamic acids.

Methods: Biocomposites were studied by IR and UV/vis spectroscopy, TPD MS, SEM, and thermogravimetry. Their biocompatibility was studied by hemolytic tests *in vitro*. To evaluate the antibacterial properties of the hybrid organic-inorganic material iodine@chitosan@silica, its interaction *in vitro* with a test strain of the conditionally pathogenic microorganism *E. coli* was investigated.

Results/Conclusions: New hybrid organo-inorganic materials based on supramolecular complexes [iodine-chitosan]@silica can be used to develop hemostatic agents similar to chitosan-based dressings used in field conditions and in disaster medicine. The obtained hybrid materials, [bioactive molecule@polysaccharide@silica/ceria], are promising for the creation transdermal drugs for the treatment of wounds, burns and skin regeneration with prolonged action, which could combine the antimicrobial properties of iodine, angioprotective properties of venoruton, hemostatic and regenerating properties of chitosan, biocompatible properties of dextran, anti-edematous and protein-absorbing properties of silica.

P2. BIODEGRADABLE SILK FIBROIN MATRICES ENHANCE RE-EPITHELIALIZATION IN A HUMAN 3D *EX VIVO* WOUND MODEL

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Background: Effective wound healing remains a clinical challenge, particularly during the re-epithelialization phase. Silk fibroin, a biopolymer derived from *Bombyx mori*, shows promising regenerative properties due to its biocompatibility, tuneable degradation, and mechanical versatility. This study aimed to evaluate the efficacy of cast and electrospun silk fibroin matrices in promoting wound closure using a standardized human 3D *ex vivo* skin model.

Methods: Silk fibroin membranes and nonwoven matrices were fabricated using PureSilk® technology and applied to partial-thickness wounds in human skin explants obtained from abdominoplasties. Wounds were cultured at an air–liquid interface for 5-20 days. Tissue regeneration was assessed immunohistologically via hematoxylin-eosin (HE), keratinocyte proliferation (Ki67), and apoptosis (TUNEL). Biocompatibility was tested *in vitro* using direct and indirect cytotoxicity assays with fibroblasts and keratinocytes according to ISO 10993-5.

Results: Both silk fibroin formats demonstrated excellent biocompatibility. Membrane-treated wounds showed a steady increase in epithelial coverage, from 69.3% on day 5 to nearly complete closure by day 15. Nonwoven-treated wounds closed more slowly but reached full closure by day 20, with notable cellular infiltration suggesting improved integration and future degradation. In contrast, control wounds showed inconsistent healing, ultimately declining by day 20. At all time points, treated wounds outperformed controls, with membranes showing the most consistent regeneration. Ki67 staining data suggest that fibroin matrices, particularly nonwovens, can enhance keratinocyte proliferation and migration compared to non-treated controls. Apoptosis remained low and consistent across all groups, indicating a balanced regenerative response.

Conclusions: Silk fibroin membranes and nonwovens significantly enhanced wound regeneration in a human *ex vivo* model. Membranes supported rapid surface epithelialization, while nonwovens encouraged matrix integration and deeper cell infiltration. These findings demonstrate the translational potential of silk fibroin matrices as biodegradable, cell-supportive wound dressings and highlight the value of *ex vivo* human models in preclinical biomaterial evaluation.

P6. ADDITIVE MANUFACTURING OF HETEROGENEOUS BIOMATERIALS FOR THE REPAIR OF COMPLEX TISSUES

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Background: Tissue engineering is used to repair complex structures in the human body. Despite the heterogeneity of tissues, to date researchers have mostly worked on oversimplified homogenous biomaterials. Additive manufacturing is a promising technique that allows the production of heterogenous materials by combining various types of inks. The aim of this study is to create a heterogeneous material bioinspired by the tooth, using silk-based inks. The resulting structure combines a soft foam core with a hard-shell structure to mimic of native tissues.

Methods: Silk cocoons from the *Bombyx Mori* worm were boiled, dissolved and dialyzed to obtain a silk fibroin solution. Ceramic, water and 20% fibroin solution were mixed to create an ink that was used to 3D print a shell structure with a homemade bioprinter. A foam solution composed of water, glycerol and 4% fibroin was added into the printed shell before freezing and lyophilization. Compression tests, microscopic observations, as well as viability and antibacterial assays were performed.

Results: Structural analysis showed a clear separation of the two structures but also a good adhesion of the foam to the hard shell. Mechanical studies demonstrated that the shell increased the rigidity of the whole material. When on this scaffold, L929 murine fibroblasts cells presented different interactions depending on the area of growth with a spreading of 100% on foam and 80% on ceramic at D7. Cytocompatibility was confirmed by measuring the viability, adhesion and proliferation of L929 fibroblasts and dental pulp stem cells.

Conclusions: Heterogeneous biomaterials, in composition but also in structure, can be complex to design and to fabricate and the technical evolution of manufacturing brought by multi-head 3D printers opens up new approaches to a better adaption to the inherent complexity of the body.

P7. DEVELOPMENT OF A PRO-ANGIOGENIC HYALURONIC ACID HYDROGEL DECORATED WITH VASCULAR CELL-DERIVED EXTRACELLULAR MATRIX

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Background: Diabetic foot ulcers are a debilitating and life-threatening complication affecting 19-34% of diabetic patients. Current treatments are insufficient to support healing due to the underlying diabetic pathophysiology. *In vitro*-generated extracellular matrix (ECM)-derived biomaterials constitute an innovative approach to wound healing, as cell-derived ECM has the biological complexity required to control cell behaviour. We hypothesise that human induced pluripotent stem cell (hiPSC)-derived vascular cells deposit ECM which enhances pro-angiogenic processes, and when combined with a hyaluronic acid (HyA) hydrogel supports wound repair.

Methods: As the source of pro-angiogenic ECM, hiPSCs were differentiated and sorted into two populations by CD31-expression: CD31-positive endothelial cells (iEC), and CD31-negative stromal cells (iSC). iSCs were characterised by SM-22α, and vimentin immunostaining. ECM deposition by iSCs was stimulated by ascorbic acid (AA) and macromolecular crowder carrageenan (C) supplementation. Following 21 days, decellularisation of iSC-deposited ECM was achieved by freeze-thaw, Triton X-100, ammonium hydroxide, water, and DNase, and confirmed by DNA quantification and DAPI staining. Initial ECM characterisation was done by immunostaining and histology. Scratch and tube formation assays determined the capacity of decellularised ECM to support angiogenic processes.

Results: Differentiation and sorting of hiPSCs was confirmed by immunostaining and flow cytometry, while iSCs were characterised by positive SM-22α and vimentin immunostaining. Treatment of iSCs with AA and C increased ECM deposition without compromising cell viability. Decellularisation was confirmed by reduced DNA concentration and negative DAPI staining. Immunostaining and Sirius red staining confirmed deposition of fibronectin and fibrillar collagens, which was maintained post-decellularisation. Media supplementation with ECM enhanced iEC migration and formation of tube-like networks.

Conclusions: The results suggest ECM generated *in vitro* by iSCs supports key angiogenic processes, specifically endothelial cell migration and tube formation. Next steps include ECM histological analysis and assessing the ECM's capacity to enhance the pro-angiogenic potential of a HyA-based hydrogel *in vitro* and *in vivo*.

P8. DEVELOPMENT OF A BILAYERED HYALURONIC ACID HYDROGEL COMBINING ANTIMICROBIAL AND PRO-ANGIOGENIC PROPERTIES FOR CHRONIC WOUND HEALING

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Background: Chronic wounds, such as diabetic foot ulcers (DFUs), represent a significant clinical and economic burden. A lack of tissue vascularisation, as well as the increasing challenge of antimicrobial resistance, limits the efficacy of conventional treatments. Natural biopolymers offer promise due to their biocompatibility and ability to mimic the extracellular matrix. However, they lack intrinsic antimicrobial properties, crucial for infection control. To overcome this, we developed a multifunctional bilayered hyaluronic acid (HyA) hydrogel with two distinct functional layers: a superficial layer with antimicrobial peptides (AMPs) for localised antimicrobial protection and a deeper layer delivering mesenchymal stem cell-derived extracellular vesicles (MSC-EVs) to support vascularisation and accelerate healing.

Methods: HyA was modified with acrylate groups (AcHyA) to allow crosslinking for hydrogel formation and biofunctionalisation. The superficial antimicrobial layer was conjugated with a cysteine-terminated AMP, PP4-3.1, and gelatin to support cell adhesion and antimicrobial properties against common pathogens, *Staphylococcus aureus* and *Escherichia coli*. For the deeper layer, MSC-EV bioactivity was first confirmed using 2D endothelial cell (EC) migration assays, followed by loading into a softer, more rapidly degrading HyA hydrogel. We characterised the biophysical properties of each layer (gelation, stiffness, swelling behaviour, and enzymatic degradation).

Results: The superficial layer gelled within 5 minutes, with a storage modulus of ~1000 Pa. Gelatin conjugation enhanced cell adhesion without compromising the integrity of the hydrogel. AMP conjugation conferred bactericidal activity against both pathogens. The deeper layer gelled within 5 minutes and showed lower stiffness (~450 Pa). In 2D, EV treatment significantly increased EC migration, confirming their pro-angiogenic potential. Both layers gelled rapidly, facilitating the formation of a stable bilayered structure with distinct layers visible.

Conclusions: The bilayered AcHyA hydrogels show promise as a platform to combine antimicrobial and pro-angiogenic approaches in a spatiotemporal manner. Future work will optimise EV loading and evaluate hydrogel performance in chronic wound models.

P11. MODULAR VISIBLE LIGHT ACTIVATED NATURAL HYDROGEL BIOCONJUGATION SYSTEM

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Background: Hydrogels are widely used in cell encapsulation, tissue engineering, regenerative medicine, and drug delivery due to their customizable biochemical and material properties. However, the choice of biopolymer remains a key limiting factor, as different biopolymers exhibit diverse physiochemical characteristics. To overcome these limitations, we developed a modular natural hydrogel bioconjugation system using polyphenol radical chemistry activated by visible blue light and a natural vitamin photosensitizer.

Methods: Our system enables rapid and cell-safe bioconjugation by mixing desired biomolecules (e.g. ECM proteins, growth factors, cytokines, peptides, nucleic acids) with polyphenol-modified glycosaminoglycans (GAGs) and a natural vitamin photosensitizer, followed by brief exposure to 465 nm blue light. This results in the covalent integration of biomolecules onto the GAG backbone, allowing us to design a library of customizable matrices. We validated the blue light-mediated coupling chemistry using NMR spectroscopy, liquid chromatography, mass spectroscopy, and gel electrophoresis techniques. We next evaluated biological functions with encapsulated colorectal cancer cells in various HA-protein hydrogel matrices.

Results: The novel bioconjugation chemistry is versatile, rapid, and customizable for diverse biological applications. Using polyphenol-modified hyaluronic acid (HA-GA) as a model GAG, we demonstrated efficient conjugation with proteins with varying charges and isoelectric points along with different nucleic acids. The mechanics and stiffness of the 3D matrix can be tuned by optimizing the photosensitizer amount or blue light exposure time. We demonstrated the biological functionality of HA-protein hydrogels by encapsulating HCT116, a human colon cancer cell line, and cells responded differently in contact with various ECM proteins. Lastly, encapsulated HCT116 Wnt reporter cells increased luciferase signal significantly after crosslinking.

Conclusions: This innovative bioconjugation system shows strong potential for enhancing the biological functionality and tissue specificity of hydrogels across a wide range of applications. The technique is easy to use, cell-safe, and user-friendly.

P12. SLAM-BASED 3D PRINTED HYDROGELS WITH HYPERELASTIC PROPERTIES FOR SKIN SUBSTITUTE DESIGN

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Background: Natural-synthetic composite hydrogels offer potential for replicating the nonlinear and viscoelastic mechanical behaviour of full-thickness human skin. These properties arise from the hierarchical and anisotropic architecture of the epidermis and dermis. This study aims to develop a photo- and ionically crosslinked PEGDA-based multi-component hydrogel system fabricated via Suspended Layer Additive Manufacturing (SLAM), and to characterize its mechanical behaviour using hyperelastic modelling for applications in skin tissue engineering.

Methods: A composite hydrogel was formulated using sodium alginate crosslinked with $CaCO_3$ and glucono-δ-lactone (GDL) for slow ionic gelation, gelatin for biocompatibility, and varying concentrations of polyethylene glycol diacrylate (PEGDA) (2–12% w/v) to tune elasticity. Uniaxial compression tests were conducted at a fixed strain rate of 0.1/s and fitted to five constitutive models (Mooney-Rivlin, Ogden, Neo-Hookean, Fung, Gasser-Ogden-Holzapfel) to quantify nonlinear responses. Rheological analysis was conducted to evaluate shear-thinning, and SLAM was used for 3D printing within a 0.5% agarose bath using a 25G needle and a six-line grid structure with 15 mm × 3 mm spacing. Bilayer constructs with 40% overlap were printed to assess interlayer adhesion.

Results: Higher PEGDA concentrations increased hydrogel stiffness, aligning with the mechanical profile of porcine dermis. Mooney-Rivlin and Ogden models showed excellent fit ($R^2 > 0.98$), capturing the nonlinear behaviour. The hydrogel exhibited shear-thinning behavior ($n = 0.4853$, $K = 3.609$; $R^2 = 0.9905$), confirming the suitability for SLAM printing. Cohesive failure away from the interface indicated strong interlayer adhesion.

Conclusions: The SLAM-printed PEGDA-alginate-gelatin hydrogels demonstrate strong potential as mechanically tunable skin-mimicking materials. Their nonlinear mechanical response and robust interlayer adhesion provide a promising basis for future multilayer skin substitutes. Next steps include incorporating cellular components and additional layers to replicate full-thickness tissue.

P13. A THERANOSTIC APPROACH TO UNDERSTANDING HEALING PROGRESSION IN PROBLEM WOUNDS

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Background: There is considerable interest in the use of soluble protein signals released by the cells involved in the wound healing process, biomarkers, to support current methods of clinical wound healing assessment. The measurement of particular biomarkers indicated to be present or changed in concentration levels during phases and events in the wound healing process should inform clinical decisions and potentially provide a quantifiable output for healing/non-healing (Longfield, 2023). Neotherix has developed a wound theranostic system (RegeniTherix™), consisting of an electrospun polymer scaffold and thermoswitchable hydrogel to capture released biomarkers. The gel can then be sampled and applied to an appropriate diagnostic technology for biomarker detection and measurement.

Methods: The scaffold component was electrospun from polyglycolic acid (PGA). The hydrogel was produced from Poloxamer P407. Both components were terminally sterilised (using gamma irradiation and electron beam respectively). Proof of concept was achieved through application of scaffold and gel to a porcine wound model. Recovery and measurement of model biomarkers from the hydrogel component was demonstrated using a Guided Mode Resonance (GMR) sensor-based diagnostic system (Kenaan, 2020).

Results: The RegeniTherix PGA scaffold-Poloxamer hydrogel system was shown to be biocompatible and conducive to healing in the porcine model. In separate experiments, IL-6 and TNF-α were added to simulated wound exudate as model biomarkers and absorbed into the hydrogel component. Following adjustments to overcome interference with the GMR system, measurement was shown to be achievable and was demonstrated to be within the clinically relevant range of sub-ng/mL to µg/mL concentrations.

Conclusions: This work and other recent publications (Saberainpour, 2023, Longfield, 2023, Rippon, 2024), support the concept that multiplexed measurement and monitoring of a set of biomarkers will guide clinical decision-making for the management of wounds. Some aspects of this work are published as Bakshi (2023).

P15. SCIENTIFIC CRITERIA DEMONSTRATING FORM-STABILITY AND CONTINUOUS WOUND COVERAGE OF *IN SITU* BUILT WOUND DRESSINGS

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Background: The classification of wound care products remains complex due to the absence of clear, quantifiable criteria distinguishing in situ form-stable wound dressings from hydrogels, creams, or ointments. *In situ* form-stable dressings act as physical constructs that maintain their shape upon application, providing structural coverage and supporting wound healing. In contrast, viscous formulations such as hydrogels, creams, and ointments deliver moisture or active substances through a concentration gradient. This study proposes the use of bioengineering and rheological principles to define material-based criteria that differentiate these categories, focusing on viscoelastic behaviour and solid-like matter content.

Methods: Rheological testing offers a functional method to distinguish between form-stable and viscous wound care products. Oscillatory dynamic shear testing measures the storage modulus (G′) and loss modulus (G″), which represent the elastic (solid-like) and viscous (liquid-like) components of a material, respectively. From these, the phase angle (tan δ = G″/G′), a dimensionless parameter, is calculated. A lower tan δ indicates a predominantly elastic material, characteristic of in situ form-stable dressings, whereas a higher tan δ reflects dominant viscous behaviour typical of hydrogels, ointments, or creams.

Results: Tube-dispensed model wound care products were evaluated for their G', G'' and tan δ. Results consistently demonstrated that *in situ* form-stable materials exhibited a high storage modulus relative to the loss modulus, resulting in low phase angles indicative of their solid-like, form-stable nature. This contrasts with the rheological profile of non-dressing formulations.

Conclusions: This study advocates the use of rheological metrics as a quantitative and objective framework for the regulatory classification of wound care products. By assessing viscoelastic properties, products can be reliably distinguished as dressings or non-dressings, independent of application mode. This approach offers a reproducible basis for product categorization, with potential implications for clearer regulatory guidance and reimbursement strategies.

P16. SURFACE FUNCTIONALISATION OF BULK METALLIC GLASS USING LASER SURFACE TEXTURING FOR ORTHOPAEDIC APPLICATIONS

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Background: Osteoarthritis afflicts 10 million people in the UK, with the only long-term treatment strategy remaining orthopaedic intervention. Bulk metallic glasses (BMGs) possess advantageous physical properties for orthopaedic applications (high wear and corrosion resistance, high elasticity), compared to traditional implant alloys. Moreover, laser surface texturing (LST) can modulate the host response, expediting biomaterial osseointegration. The aim of this research is to assess BMG surface functionalisation using LST for enhanced tissue regeneration.

Methods: LST of AMLOY-ZR02 (Heraeus), a BMG with nominal composition: $Zr_{65}Cu_{16}Ni_{12}Al_4Ti_3$, was investigated in this study. The influence of LST parameters (fluence, (E_D), scanning speed (v), pulse duration (τ)) on the topography of groove-textured specimens was assessed using scanning electron microscopy (SEM) and optical profilometry. A full-factorial design of experiments (DOE) evaluated the effect of LST on BMG surface roughness (S_a), wettability, and surface free energy (SFE), applying a 3-way analysis of variance with Tukey's post-hoc test for statistical analysis. X-ray diffractometry (XRD) assessed the post-LST surface crystallinity of the specimens.

Results: Augmentations in fluence, and reductions in scanning speed and pulse duration increased material ablation and redeposition of ejected melt-material during texturing. The DOE identified that S_a was significantly influenced by all laser input parameters ($P<0.001$). AMLOY-ZR02 wettability reduced with decreasing pulse duration ($\Delta\mu = 8.9\%$, $P<0.01$) and increasing fluence ($\Delta\mu = 14.5\%$, $P<0.001$), the latter also reduced SFE at $\tau = 220$ ns ($\Delta\mu = 59.6\%$). XRD identified that LST retained the amorphous BMG molecular structure.

Conclusions: Nanosecond LST produces defined surface topographies on BMG biomaterials. Unexpectedly, micromachining decreased wettability and SFE despite increasing surface roughness. Literature indicates that LST influences both surface topography and chemistry; consequently, BMG surface chemistry following LST will be assessed via XPS. Protein adsorption and THP-1-derived macrophage polarisation will be quantified to further investigate the effect of LST BMGs on cellular responses and tissue regeneration.

P17. DEVELOPMENT OF TOUGH, INJECTABLE HYDROGELS FOR CARTILAGE REGENERATION AND OSTEOARTHRITIS TREATMENT

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Background: Osteoarthritis is the most common chronic join disease, affecting more than 500 million individuals or 7 % of the global population[1]. Injectable hydrogels are a class of treatment strategy for cartilage defects and osteoarthritis that have the significant benefit of eliminating the need for invasive surgeries while replacing and regenerating native cartilage. Crucial considerations of such hydrogels for implementation in load-bearing joints include adequate mechanical properties and biocompatibility. From this perspective, polyethylene glycol-acrylate (PEG-AC), chondroitin sulphate (CS) and gelatin were the components selected for the development of an injectable hydrogel to replace and regenerate cartilage due to their biocompatibility, regulatory approval, and superior mechanical properties.

Methods: Methacrylic anhydride was used to introduce methacrylate groups to CS and gelatin. Hydrogels were formed after mixing these components with PEG-AC in various ratios and crosslinking using UV light. Hydrogels were subsequently assessed mechanically using rheology, dynamic-mechanical-analysis and cyclic compression. Cytotoxicity of hydrogels and their degradation products was assessed using Prestoblue following seeding of fibroblasts and chondrocytes. Cell behaviour following seeding was investigated using RT-qPCR to quantify chondrogenic gene expression.

Results: Hydrogels and their individual components were not cytotoxic (cell survival > 85%). Hydrogels composition significantly influences their mechanical properties with PEG-AC being particularly significant.

Conclusions: Hydrogels composed of a mix of CS-MA, PEG-AC and GelMA as opposed to individual components seem to be the most attractive for use as an injectable cartilage replacement. These hydrogels have potential as a treatment for osteoarthritis due to their injectability, biocompatibility and promising mechanical properties in the context of native cartilage.

P18. EFFECT OF MODIFIED MICROSCALE SURFACE TOPOGRAPHY OF TI-6AL-4V ALLOY ON HUMAN NEUTROPHIL RESPONSES

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Background: Ti-6Al-4V has been widely used in medical implants; however, the early immune response following implantation, is poorly understood. While the influence of surface topography on macrophage behaviour has been well explored, its specific effects on neutrophils remain unclear. This study aims to compare neutrophil behaviour on Ti-6Al-4V surfaces with varying microscale topographies to understand the role of surface features in early immune regulation.

Methods: Human neutrophils were isolated by centrifugation, fluorescently-labelled, and seeded on the surfaces of four groups of Ti-6Al-4V discs, previously prepared by polishing (P-smooth and P-rough) or sandblast acid-etching (SLA 250 and SLA50). The cells were incubated for 3, 5, 15, 30, and 60 minutes, respectively, and fixed. The physicochemical properties of the discs were characterised; and confocal laser scanning microscopy allowed simultaneous observation of cell morphology and surface topography. Assessments of cellular ROS release and NETs formation are currently ongoing.

Results: SEM showed unidirectional micro-grooves on P-Rough surfaces and irregular pits on SLA surfaces (Fig. 1). SLA250 surfaces were the roughest (Ra≈1.63 μm), followed by SLA50 and P-Rough (Ra≈0.84 μm), with P-Smooth being the smoothest (Ra≈0.04 μm). Cell analysis showed a decreasing cell area on SLA250 after 5 min, while SLA50 remained stable. Cells adhered to P-Rough surfaces had the smallest cell area, especially at 30 min, while P-Smooth had larger cell spreading. Cell numbers increased over time in all groups. Cells on P-Rough exhibited time-dependent elongation aligned with the groove direction, while on irregular surfaces (SLA50, SLA250, and P-Smooth) showed no significant change.

Conclusions: Ti-6Al-4V distinct microscale surface topography influenced neutrophil adhesion and morphology at early timepoints. The directionality of surface grooves had a contact-guiding effect on neutrophils. These findings suggest that surface design affects early neutrophil immune responses.

P19. . PURIFIED KERATAN SULPHATE SUPPORTS THE EXPANSION OF HUMAN INDUCED PLURIPOTENT STEM CELLS INTO EYE-LIKE LINEAGES

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Background: Human induced pluripotent stem cells (hiPSCs) can be expanded into Self-formed, Ectodermal, Autonomous, Multi-zones (SEAMs) - small circular colonies of cells with four concentric zones. Cells in each zone resemble cells of different tissues of the eye (Hayashi *et al., **Nature*** 2016;531:376-80). The substrate on which hiPSCs are grown is also known to influence the nature of the resultant SEAM, with the glycoprotein, laminin, particularly influential (Shibata *et al.*, ***Cell Rep*** 2018;25:1668-79). Here, we investigate whether or not the glycosaminoglycan, keratan sulphate (KS), affects hiPSC growth and SEAM formation.

Methods: Ten porcine corneas were subjected to guanidine hydrochloride chaotropic extraction, digestion and dialysis. After density gradient centrifugation and separation of proteoglycans and glycosaminoglycans by HPLC using ion exchange and size-exclusion columns, purified KS was used to coat cell culture plates (in conjunction with laminin 511) at concentrations of 0 μg/cm^2 (control group), 0.25 μg/cm^2, 0.5 μg/cm^2 and 1.0 μg/cm^2. hiPSCs were cultivated into SEAMs on these plates (n=3 at each concentration).

Results: KS was obtained from porcine corneas with a purity of over 90%, and was able to support SEAM growth at all coating concentrations used. Notably, at day 10 of culture, hiPSCs grown on 0.5 μg/cm^2 high-sulphated KS consistently generated more than 90 SEAMs/dish, more than for all other experimental conditions. At the 10-day juncture, immunofluorescence microscopy and qPCR revealed an elevation the transcription factor protein, Oct4, on KS-coated surfaces. SEAMs grown for 4-weeks on high-sulphated KS, moreover, showed high gene expression levels for PAX6, a master regulator of eye development, and p63, an indicative corneal epithelial stem cell marker. Immunohistochemistry further revealed increased numbers of p63/PAX6-positive hiPSC-derived corneal epithelial-like cells in the region of the SEAM (zone 3) that gives rise to surface ectoderm.

Conclusions: KS is able to enhance the formation of hiPSC-derived eye-like SEAM constructs.

P20. LABEL-FREE ANALYSIS OF INDIVIDUAL EXTRACELLULAR VESICLES USING PHOTO-INDUCED FORCE MICROSCOPY

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Background: Extracellular vesicles (EVs) are cell-derived phospholipid spheres, carrying proteins, nucleic acids and metabolites, playing key roles in intercellular communication, cancer progression and tissue repair. However, conventional EV characterisation methods assume homogeneity and lack the resolution to analyse individual EVs. Photo-induced force microscopy (PiFM), which integrates infrared (IR) spectroscopy with atomic force microscopy, enables topographical and chemical mapping at <10nm resolution, offering a novel approach to EV analysis. This study aimed to develop PiFM-based methods for single-EV analysis and compare IR signatures of EVs from human bone marrow stem cells (hBMSCs) and prostate cancer cells (Du145).

Methods: Gold-patterned silicon wafers, fabricated via photoresist patterning and e-beam deposition, were functionalised with (3-aminopropyl)triethoxysilane (APTES) to immobilise EVs. Surface modification was confirmed by quartz crystal microbalance, water contact angle and X-ray photoelectron spectroscopy (XPS). EVs were isolated and characterised using nanoparticle tracking analysis, cryo-electron microscopy, flow cytometry and western blotting, and incubated on the surfaces for 1 hour before being rinsed three times with ultrapure water and analysed using PiFM.

Results: APTES-functionalised gold surfaces demonstrated an increase in Saurbrey mass (2000-3000ng/cm^2); an increase in water contact angle (7.1±1.8° to 75.2±0.6°); and an increase in C1s, O1s, Si2p peaks and a decrease in Au4f XPS peaks. EVs (~100nm) expressed CD81, CD63, CD9, TSG101 markers and lacked cellular contaminants (Calnexin, Apolipoprotein A). PiFM analysis revealed distinct IR peaks for lipids (1800–1700cm^{-1}), proteins (1700–1500cm^{-1}, 1400–1200cm^{-1}) and nucleic acids (1717-1700 cm^{-1}), with compositional differences observed between EVs from different cell types, within the same sample and across individual vesicles. Protein secondary structure analysis showed hBMSC EVs had balanced α-helices, β-sheets and random coils, whilst Du145 EVs exhibited reduced α-helices and increased β-sheets/β-turns.

Conclusions: This is the first study to demonstrate the ability of PiFM to resolve nanoscale heterogeneity in EVs, revealing structural biomarkers that may distinguish regenerative from malignant EV populations.

P21. NEUROPROTECTIVE AND NEUROREGENERATIVE EFFECTS OF SMALL EXTRACELLULAR VESICLES SOURCED FROM VARIOUS MESENCHYMAL STEM CELLS

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Background: Retinal Ganglion Cells (RGCs) are responsible for relaying visual signals to the brain, with their loss in diseases like glaucoma resulting in irreversible blindness. Small Extracellular Vesicles (sEVs) are nanocarriers that participate in cell-to-cell communications. Although their ability to preserve injured RGCs is well-established, their cargo composition, and thus their efficacy, vary greatly depending on the type and circumstances of the parental cells. Here, we compared the neuroprotective and regenerative potential of sEVs from multiple cell lines and used historical data of sEV cargo to investigate their mechanism of action.

Methods: sEVs were isolated from conditioned media and examined using ZetaView nanoparticle tracking analysis. Dissected retinae of Sprague-Dawley rats were cultured on Laminin-coated chamber slides with different concentrations of sEVs for 3 days (N=3). Anti-β-III-Tubulin staining was performed and visualised using fluorescent microscopy. Cell survival rates and neurite growth were assessed using ImageJ and GraphPad Prism (ANOVA). Historical data of sEV miRNA cargo were collected and analysed using GraphPad Prism (ANOVA) and Ingenuity Pathway Analysis.

Results: Most vesicles had a median size of 130-150 nm. Adipose and Bone Marrow MSCs-sEVs increased cell survival rates 3-4-fold, while the others did not have a noticeable effect. Similar results were observed for the number and the length of neurites. *In silico* analysis identified significant differences in the cargo and highlighted pathways in which sEVs could elicit their therapeutic effects.

Conclusions: Adipose and, to a lesser extent, Bone Marrow MSC sEVs were found to be the most potent and dose-dependent. Other cell types did not show significant differences in cell survival or neurite growth. sEVs were shown to be promising candidates for optic nerve protection and regeneration. Further analysis of their cargo and the affected biochemical pathways could deepen our understanding of RGC degeneration.

P22. IMMUNOHISTOCHEMISTRY AND VOLUME ELECTRON MICROSCOPY OF DEVELOPING HUMAN LACRIMAL GLANDS AND LACRIMAL-GLAND-LIKE ORGANOIDS DERIVED FROM HUMAN INDUCED PLURIPOTENT STEM CELLS

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Background: Dry eye disease, characterised by instability of the tear film at the corneal surface, affects millions of people worldwide, particularly the elderly, and can be sight-threatening. The condition is driven by dysfunction of the meibomian and/or lacrimal (tear) glands (LG), yet no effective replacement therapy currently exists. In a recent study, Hayashi and associates (*Nature* 2022; 605:126–131) successfully engineered LG-organoids from human induced pluripotent stem cells (hiPSCs), which restored tear production in rats following transplantation. Here, we aim to compare the *in vitro* formation of LG-organoids to the development of human foetal and adult LGs.

Methods: Human foetal LGs (8–21 post-conception weeks) were obtained from the Human Developmental Biology Resource, with adult tissue from a 61-year-old donor sourced via the National Disease Resource Interchange (Philadelphia, USA). LG-organoids were grown from cells isolated from hiPSC-derived SEAMs (Self-formed, Ectodermal, Autonomous, Multi-zones) and harvested at 7, 10, 14, and 21 days. All tissues were fixed in aldehyde solutions and prepared for 3D imaging using serial block-face scanning electron microscopy (SBF-SEM) and immunofluorescence microscopy to localise key developmental and functional markers (α-actin, CD44, PAX6, KRT12, KRT14, lactoferrin (LTF), and lysozyme (LYZ).

Results: 3D reconstructions from 500-1000 SBF-SEM serial images showed formation of secretory acini from ectodermal cell condensations with subsequent appearance of lumina, branching collecting ducts and major outflow channels both in human LGs and LG-organoids. Confocal fluorescence microscopy also revealed similarities, notably in staining patterns of acinar cell CD44, PAX6, KRT14, with α-actin prominent in myoepithelial cells and unusual non-uniform labelling of KRT12. Staining for tear-film proteins, LTF and LYZ, was lacking in pre-functional LG-organoids *in vitro*.

Conclusions: Cellular ultrastructure, including organelle polarity and glandular organisation, namely lobules and ducts, and immunostaining; were remarkably similar between LG-organoids grown from hiPSCs and human LGs across foetal stages and adult tissue.

P24. MICROBIAL LANDSCAPES OF WOUNDS: COMPARING COMMUNITY COMPOSITION ACROSS CHRONIC AND ACUTE SKIN INJURIES

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Background: Non-healing wounds represent a substantial clinical and economic burden, accounting for approximately 4% of the NHS budget and up to half of community nurse time. Wound microbiota typically exist as biofilms, a lifestyle that promotes microbial persistence, horizontal gene transfer, and antimicrobial tolerance. Within these biofilms, microbial and host interactions, both synergistic and antagonistic, shape community structure and influence infection outcomes. While pathogens such as *Pseudomonas aeruginosa* and *Staphylococcus aureus* are frequently associated with wound infections of all types; to what extent the microbial communities vary across wound types remains unclear.

Methods: We performed a meta-analysis of 275 published studies (1969–2023) reporting microbiological data from five wound types: chronic wounds (diabetic foot ulcers, pressure ulcers, venous leg ulcers), burn infections, and non-surgical traumatic skin wounds. The analysis revealed that microbial communities in burns and chronic wounds were more diverse than those in traumatic wounds.

Results: Across all wound types, common bacterial species included members of the highly antimicrobial-resistant ESKAPE(E) group (e.g., *P. aeruginosa*, *Escherichia coli*, *S. aureus, Acinetobacter baumannii*), along with environmental and commensal human microbiome organisms. The presence of such organisms strongly suggests that wound colonisation was likely via contamination from the local environment, pets, the patient themselves or their carers, and not via a “traditional” nosocomial transmission route. Anaerobic and fungal species were frequently detected, though fungi, particularly *Candida* spp.; were only highly enriched in burn wounds.

Conclusions: These findings highlight shared and distinct microbial features across wound types and underscore the complexity of wound biofilms. The data will inform our ongoing laboratory research focused on interspecies interactions in polymicrobial biofilms and guide rational microbial selection for *in vitro* models. This evidence-based approach supports the development of targeted strategies to disrupt biofilms and improve wound healing outcomes.

P26. CATION-ADJUSTED END-POINT BUFFER ALTERS BACTERIAL BIOFILM CHARACTERISATION WITH CONFOCAL IMAGING AND THE CRYSTAL VIOLET ASSAY

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Background: Chronic wounds are an escalating global health concern, with annual NHS costs reaching £8.3 billion. Bacterial biofilm-associated infections contribute significantly to wound persistence, where pathogens exist in organised communities encased in an extracellular polymeric matrix, which resists antibiotic treatment and immune clearance. Accurate *in vitro* characterisation of bacterial biofilms is crucial for effective evaluation of antimicrobial drugs. The effect of the end-point buffer on standard *in vitro* biofilm characterisation (for washing and maintenance of sample hydration) has not been previously assessed. In this study, we examined the effect of cation adjustment of calcium and magnesium ions in end-point buffer on the structural properties of bacterial biofilms.

Methods: Biofilms of common wound pathogens, *Pseudomonas aeruginosa* PAO1, Methicillin-resistant *Staphylococcus aureus* (MRSA) 1004A and *Escherichia coli* IR57 were grown for 24 h (37°C, 20 rpm). Standard end-point phosphate-buffered saline (PBS) containing calcium (0–50 mg/L) and magnesium (0–25 mg/L) was applied to the biofilms before quantifying their biomass and structural properties using the crystal violet assay and confocal laser scanning microscopy (CLSM) imaging, with LIVE/DEAD and matrix staining, and COMSTAT image analysis.

Results: Distinct differences in *P. aeruginosa* and MRSA biofilm structures were apparent depending on the PBS-cation concentrations used. This was exemplified by significant decreases in biofilm thickness and cell viability after application of standard PBS compared to cation-adjusted PBS ($P < 0.05$) in the CLSM assays. This result was reflected in *P. aeruginosa* biofilm biomass decreases observed in the crystal violet assays. In contrast, simple biofilm rinsing (<5 mins contact time) with different PBS buffers demonstrated no apparent difference in biofilm biomass.

Conclusions: This study demonstrates that end-point buffer can have a significant effect on 3-dimensional biofilm structure and therefore cation-adjustment should be considered for *in vitro* biofilm modelling and testing of antimicrobial agents.

P27. THE ACUTE PHASE RESPONSE TO BURN INJURY: AN *IN-SILICO* MODELING APPROACH

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Background: Burn injuries cause complex events, such as acute inflammation, which play a crucial role in tissue repair and regeneration. The activation of the complement system after burn injury is essential to the inflammatory response and mediates various immune pathways during healing. Our objectives were to develop and validate a computational model that simulates the acute inflammatory phase during the first 18 days post burn injury by using animal data.

Methods: Our Agent Based Cellular Potts model approach includes different cell types as individual agents, cytokines and growth factors that interact within a defined tissue environment on a two-dimensional wound simulation. This model considers systemic factors such as the concentration of cytokines and chemokines immune cell recruitment, and local factors including Damage Associated Molecular patterns that signal tissue damage. We incorporated experimental data from animal burn models from the literature to validate the interactions of key players within the acute inflammation cascade, seeking to create a representation of complement activation, inflammatory events and the associated consequences over time.

Results: Through simulation, we investigated how different factors, such as the severity of burn injury, the prolonged inflammation, and changes in the concentration of complement factors, affect the dynamics of the acute inflammatory phase. Furthermore, we explored the interaction between complement activation and other signalling pathways involved in burn wound healing, such as IL-6, IL-8, IL-1β, TNF-α and TGF-β1 concentration with increasing CRP concentration (in blood and wound) and complement activation.

Conclusions: This computational model provides insight into the spatio-temporal dynamics of acute inflammation driving factors after burn injuries.

P28. DECODING EARLY CELLULAR RESPONSES TO INJURY FOR ENHANCED TISSUE REGENERATION

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Background: Our work shows the intricate relationship between the early injury response, and the progression and outcome of the healing process. We discovered a wound healing marker: the phosphorylated ribosomal protein S6 (p-rpS6) that has enabled us the detailed characterization of numerous cellular fates in regeneration.

Methods: In this project, we have used several state-of-the-art wound healing models that bridge the gap between bedside care and preclinical research including:

-*in vivo* pig experiments including different types of wounds in settings mimicking the clinical scenario.

- a porcine *ex vivo* model of injury that enabled us usage of various drugs to mechanistically modulate signalling pathways involved in wounding responses.

All these experiments were evaluated phenotypically, morphologically and histologically.

Results: We have shown that the p-rpS6 protein can be used as a marker of skin tissue response to injury, forms within minutes and stays until wound healing is complete. The phosphorylation establishes as a zone, the “p-rpS6-zone”, that reaches from the injury into the tissue and delineates where the wound response is taking place. This zone is mTOR-dependant and can only form with oxygen available. The follow-up work has revealed the signalling events involved in the response to injury that determine a wide range of cell fates. Finally, we shed some light into understanding how these signalling pathways can be leveraged to improve healing.

Conclusions: The p-rpS6 zone is a powerful tool to characterize the injury responses of skin. We have made progress in elucidating the underlying molecular pathways of wound healing and propose drug treatments to activate or diminish specific cellular responses. Future steps include translation into human skin and the development of applications, such as detection devices or novel drug testing platforms, to gain further clinical relevance.

P30. REGULATION OF CXCL1 AND 8 PRODUCTION IN KERATINOCYTES AND ERK-DEPENDENT EFFECTS ON MIGRATION

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Background: Epidermal keratinocytes maintain the skin barrier and promote re-epithelialization following injury. Acute inflammation aids tissue restoration, but sustained inflammation contributes to skin disease and hinders wound healing. Thus, fine regulation of inflammatory mediator release in keratinocytes is essential for skin homeostasis. Keratinocytes can produce several chemokines, including CXCL1 and CXCL8 (interleukin-8), which are known to enhance cell migration through the receptors CXCR1 and CXCR2. Their effects on the keratinocytes themselves however remain incompletely characterized. Therefore, our objective was to uncover how CXCL1/8 production is regulated in keratinocytes and establish their effects on keratinocyte wound-healing capacities.

Methods: We used a keratinocyte cell line (HaCaT) and primary human epidermal keratinocytes (HEKa). Expression and release of CXCL1 and 8 were measured using Western blotting and ELISA. Pathway activation was followed using Western blotting, and migration was assessed by time-lapse imaging. Key proteins were inhibited pharmacologically and using silencing.

Results: Both HaCaT and HEKa cells continuously produced CXCL1/8, which was upregulated by the inflammatory cytokine TNFα as early as 1h following stimulation. The effect was sustained, lasting at least for 24h. TNFα-induced CXCL1/8 release required the transcription factor NFκB, and TRAF2 and NCK-interacting protein kinase (TNIK), a proposed upstream regulator of NFκB. TNFα also augmented migration, but the CXCR1/2 inhibitor Reparixin did not affect this. CXCL1 and 8 induced rapid activation of ERK1/2 in keratinocytes and augmented migration through CXCR1 and 2. Finally, keratinocyte migration induced by TNFα or CXCL1/8 was prevented by the ERK1/2 inhibitor, PD184352.

Conclusions: We identified TNIK as a new regulator of CXCL1 and 8 release from keratinocytes. This kinase could be a potential drug target for reducing chemokine levels. We also showed that CXCR1/2-induced ERK activation was required for accelerated keratinocyte migration. Our ongoing studies are exploring effects on other keratinocyte functions.

P31. IDENTIFYING RHOA REGULATORS IN KERATINOCYTES AND UNCOVERING THEIR ROLES IN MIGRATION, DIFFERENTIATION AND SECRETION

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Background: Epidermal keratinocytes proliferate, migrate and differentiate to re-epithelialize the wound. They also secrete soluble factors to orchestrate responses. The small GTPase RhoA was found to be important for the above processes. However, the mechanisms regulating RhoA in the context of these keratinocyte functions remain poorly defined. The overall objective of this study was to uncover how RhoA is regulated in keratinocytes and characterize RhoA activator guanine nucleotide exchange factors (GEFs) controlling migration, differentiation and secretion.

Methods: HaCaT cells and primary adult Human Epidermal Keratinocytes (HEKa) were grown in 2D and 3D cultures. Affinity precipitation with GST-RhoA(G17A) that has high affinity to active GEFs was followed by mass spectrometry or Western blotting. RhoA activity was measured using affinity precipitation or immunofluorescence with a GTP-RhoA antibody. Proteins were silenced using siRNA. Keratinocyte migration was followed using live imaging. Secretion was measured using Elisa. Keratinocyte differentiation markers were detected using Western blotting and immunofluorescence.

Results: The RhoA G17A precipitation screen identified 10 candidate GEFs that were active in keratinocytes. Of these we showed that p115RhoGEF (ARHGEF1), GEF-H1 (ARHGEF2) and Larg (ARHGEF12) were activated by Vascular Endothelial Growth Factor A (VEGF-A). VEGF-A-induced GEF-H1 activation required VEGF receptor 2 (KDR) and ERK1/2. Silencing of GEF-H1 strongly reduced VEGF-A-induced RhoA activation and slowed basal and VEGF-A -stimulated migration. GEF-H1 was also required for secretion of several chemokines, interleukins, and VEGF-A. Finally, RhoA was activated and phosphorylated GEF-H1 was elevated in the epidermis of a skin inflammatory mouse model.

Conclusions: We identified several active GEFs in keratinocyte and showed that the GEF-H1/RhoA axis is critical for migration and secretion. Ongoing studies are exploring the role of GEF-H1 in differentiation. Systematic exploration of the role of candidate GEFs in keratinocyte biology will potentially identify new ways to specifically target keratinocyte functions.

P32. ANTI-MICROBIAL AND ANTI-BIOFILM PROPERTIES OF NATURAL COMPOUNDS DERIVED FROM FRANKINCENSE (*BOSWELLIA FREREANA*) VERSUS CHRONIC WOUND BACTERIA

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Background: Non-healing chronic wounds represent significant causes of patient morbidity and financial burden to Healthcare Services. A complicating factor to impaired wound healing is microbial colonization, where bacterial species exist in polymicrobial biofilm communities within an extracellular polymeric substance matrix. Management of biofilm-associated infections represent significant challenges, as bacteria demonstrate increased resistance to antibiotic/antimicrobial therapies. Thus, the development of novel anti-microbial therapies against chronic wound infections are much needed, with natural phytochemicals acting as promising sources of potential anti-microbial biomolecules. Therefore, this study assessed the anti-microbial/anti-biofilm properties of resin and essential oil extracts and compounds purified from traditional medicine, *Boswellia frereana* frankincense; versus common chronic wound bacteria.

Methods: *Boswellia frereana* resin and essential oil extracts were prepared by solvent extraction, with chemical constituents identified by Gas Chromatography-Mass Spectrometry and High-Performance Liquid Chromatography. The anti-microbial and anti-biofilm activities of these extracts and compounds were evaluated against commercial and clinical isolates of *S. aureus* (ATCC29213, 10029, 10109), *methicillin-resistant S. aureus* (NCTC13373, 10049, 10099), and *P. aeruginosa* (ATCC27853, 10069, 10079), by Minimum Inhibitory Concentrations (MICs), Minimum Bactericidal Concentrations (MBCs); and biofilm disruption using Dead/Live staining, Confocal Laser Scanning Microscopy and COMSTAT analysis.

Results: Chemical analyses identified triterpene, epilupeol, as the main resin component, with essential oil extracts rich in monoterpenes, α-pinene and β-pinene. Essential oil extracts, α-pinene and β-pinene exhibited the greatest MICs, MBCs and biofilm disruption against *S. aureus* and *MRSA*, significantly decreasing biofilm thickness ($p<0.001$-0.01) and increasing bacterial Dead/Live ratios ($p<0.001$). However, these extracts and compounds were ineffective against *P. aeruginosa* ($p>0.05$).

Conclusions: Essential oil extracts from *Boswellia frereana* frankincense possess potent anti-microbial and anti-biofilm effects against Gram positive, *S. aureus* and *MRSA*, largely attributable to α-pinene and β-pinene. However, Gram negative, *P. aeruginosa*, was unaffected. Such findings advocate further investigations into α-pinene and β-pinene as novel anti-microbial therapeutics against Gram positive, wound infections.

P34. SAFETY AND EFFICACY VALIDATION OF STIMULI RESPONSIVE SILVER HYDROGEL FOR RAPID ELIMINATION OF BACTERIAL INFECTION IN MURINE AND PORCINE WOUNDS

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Background: The drawback of current hydrogels includes suboptimal bacterial targeting in wounds, insufficient antimicrobial release and chronic toxicity often associated with silver-based products. Smart materials that provide targeted antimicrobial delivery together with healing properties present a promising opportunity to address the increasing threat of bacterial infection. This study aimed to develop a targeted antibacterial pH/temperature responsive silver nanoparticle hydrogel that allows for triggered release of silver ions in response to changes in the wound environment.

Methods: Initial validation to demonstrate the responsive properties *in-vitro* against common wound pathogens involved material characterization, biocompatibility and release studies. Demonstration of *in-vivo* antimicrobial safety and efficacy against industry standard of care (silver sulfadiazine) was achieved using preclinical murine and porcine models of wound infection.

Results: The dual-responsive hydrogel is highly sensitive to typical pH and temperature changes during development of wound infection. There is a significant, over 90%, release of silver ions in alkaline conditions (pH 7.4), with restricted release in acidic pH (5.5). This pH dependent release, and consequent antimicrobial effect, resulted in 95% elimination of pathogens *in-vitro*. This result was confirmed *in-vivo* with potent clearing of *S. aureus* in preclinical models. The developed hydrogel was demonstrated to be safe and showed no toxicity. Murine models showed faster reepithelization and improved early collagen deposition (n=8/treatment group) whilst testing in the porcine model additionally examined early scar formation and found the treatment to be equivalent to industry standard of care in antimicrobial and wound healing capacity (n=4/treatment group).

Conclusions: The developed multifunctional hydrogel presents a promising bacteria responsive delivery platform that serves as an on-demand carrier; not only reducing side effects but also increasing antibacterial efficacy based on physiological needs. It offers a great potential to improve clinical wound infection management, providing a single platform for a long-lasting application in wound management.

P36. FIBRO-IMMUNE INTERACTIONS CHOREOGRAPH WOUND REPAIR

Dasgupta B[1,2,3], Correa-Gallegos D[1,3], Haifeng Ye[3], Jin Xu[3], Shuangxi Liu[3], Subhasree Dutta[3], Markus Sperandio[1], Philipp Henneke[2], Yuval Rinkevich[3,4]

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Background: Wound healing is a complex and dynamic process regulated by the interactions between fibroblast and immune cell populations. In adult skin, fibroblast heterogeneity plays a pivotal role in orchestrating wound repair by mediating distinct molecular cascades, whereas in fetal skin, fibro-immune interactions predominantly drive regenerative outcomes. Here, we identify that proinflammatory fibroblasts regulate the molecular signals that influence the pace of wound healing by coordinating the spatiotemporal recruitment of immune cells to the wound bed.

Methods/Results: Using murine skin injury models, single-cell transcriptomics, genetic lineage tracing, ablation and gene deletion approaches, we demonstrate that alterations in immune waves impact extracellular matrix remodelling, with neutrophils and macrophages exhibiting opposing roles in fascia contraction. Our findings emphasize the critical role of fibroblast heterogeneity and immune cell dynamics in directing foetal tissue regeneration and adult scarring. Moreover, we show that modulating dysfunctional immune responses in chronic conditions, such as through neutrophil extracellular trap (NET) removal, can significantly improve the healing process.

Conclusions: These results highlight the importance of fibro-immune crosstalk and its developmental stage-specific shifts in shaping wound repair outcomes. By elucidating the sub-cellular basis of the scarring reactions in adults, this study provides a basis for therapeutic strategies aimed at improving wound healing from early life on.

P37. A SHEEP MODEL OF UTERINE WOUND HEALING AFTER CESAREAN DELIVERY: HISTOLOGICAL AND BIOMECHANICAL PROPERTIES OF UTERINE SCAR TISSUE

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Background: Cesarean delivery (CD) rates are rising worldwide. This leads to an increasing impact of uterine rupture during trials of labour in subsequent pregnancies. To date, no method has been established to optimize uterine wound healing and reduce the risk of uterine rupture. Collecting extensive tissue samples from the scarred lower uterine segment of pregnant women for fundamental research is not feasible. A sheep model was developed to examine the effects of intraoperative interventions on uterine scarring during the subsequent pregnancy. The aim of the study was to perform CD and repeat CD with hysterectomy and systematic sampling of uterine tissue in sheep. To better understand uterine wound healing, we compared the histomorphometric collagen content and the biomechanical parameters of scarred vs. unscarred myometrium.

Methods: 48 merino ewes underwent planned cesarean delivery at 95% of gestation. After at least 365 days, 40 pregnant ewes were mated again and received repeat CD with peripartum supracervical hysterectomy. Extensive sampling of uterine scar tissue was performed. For histological analysis, sections were stained with Gomori trichrome and evaluated microscopically. For biomechanical analysis, under uniaxial tensile load, the occurring force and displacement data were recorded. ‘Stiffness’, ‘yield point’, ‘ultimate strength’ and ‘rupture strength’ together with the ‘energy expended’ were documented for each sample.

Results: Repeat CD with hysterectomy and sampling were feasible in 40 pregnant ewes. 16/80 (20%) of the uterine scars were dehiscent, defined as a macroscopic thinning of the uterine wall. Histological analysis showed notable fibrosis of the myometrium after CD. Preliminary analysis indicated different biomechanical properties of scarred compared to unscarred uterine tissue.

Conclusions: This translational model allows a standardized approach to evaluate uterine scarring during pregnancy after CD. It can help to assess the effect of intraoperative approaches to improve uterine wound healing with the aim of reducing the risk of uterine rupture in humans.

P38. TGF-B RECEPTOR I INHIBITORS IN MUSCLE FIBROSIS: MYOFIBROBLAST DIFFERENTIATION AND MYOTUBE FORMATION *IN VITRO*

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Background: Fibrosis frequently occurs in muscle wounds after trauma or surgical reconstructions, ultimately leading to suboptimal function. For instance, the surgical reconstruction of the palate in cleft palate patients leads to fibrosis in the muscles of the soft palate. This causes speech problems later in life. This study investigates the effects of TGF-βRI inhibitors AZ12799734, Galunisertib, and SM16, on myofibroblast differentiation and myotube formation.

Methods: Primary human gingival fibroblasts were treated with TGF-β1 (0, 1, 5, and 10 ng/mL) to induce myofibroblasts. Then, fibroblasts were incubated with TGF-βRI inhibitors (0, 1, 5, 10, and 20 μM) together with 10 ng/ml TGF-β1. Myofibroblast marker expression was assessed using RT-PCR (day 3), while myofibroblast differentiation was analyzed by immunofluorescence staining for α-SMA (day 6). C2C12 myoblasts were also cultured with TGF-βRI inhibitors, and gene expression (day 3) and myotube formation (day 6) were analyzed.

Results: TGF-β1 (10 ng/mL) increased the proportion of myofibroblasts from 9.3±3.5% to 38.1±4.4% ($p<0.05$), which was reduced by all TGF-βRI inhibitors even at 1 μM (for example, Galunisertib: 23.5±2.1% ($p<0.05$)). All inhibitors reduced ACTA2 and COL1A1 gene expression, while only AZ12799734 and SM16 inhibited Ki-67 expression. In C2C12 cultures, AZ12799734 and SM16 reduced the fusion index, whereas Galunisertib did not. Moreover, only Galunisertib increased myotube size from 0.09±0.01 to 0.13±0.01mm^2/nucleus ($p<0.05$). Galunisertib inhibited MyoD gene expression (at 20 μM), but not MyoG nor MyHC.

Conclusions: In conclusion, Galunisertib may have potential for improving muscle wound healing following trauma or surgical reconstructions. Further studies will focus on developing a 3D muscle fibrosis model containing human oral fibroblasts and myoblasts, which can be used to test antifibrotic drugs.

P39. PROTEIN KINASE C AND DOWNSTREAM SIGNALLING PATHWAYS MODULATED BY INGENOL MEBUTATE TO REGULATE MYOFIBROBLAST BEHAVIOUR AND SCAR TISSUE RESOLUTION

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Background: Ingenol mebutate (IngM) is a naturally-sourced, small molecule with known anticancer properties, mediated by protein kinase C (PKC) activation, especially PKC-δ. In addition to its tumoricidal capabilities, topical IngM treatments also demonstrate exceptional scar resolution properties. Our previous studies have demonstrated that IngM modulates myofibroblast behaviour to promote scar resolution by enhancing extracellular matrix (ECM) turnover, reducing type I collagen accumulation and promoting high molecular weight hyaluronan synthesis. Therefore, this study aimed to identify the kinases and downstream signalling pathways responsible for mediating these anti-scarring effects in TGF-β_1-stimulated, dermal and keloid fibroblasts.

Methods: Dermal (DF) and keloid fibroblasts (KF) were treated with TGF-β_1 (10 ng/mL) alone or with IngM (0.1 µg/mL). Kinase activity was profiled using PamGene STK Arrays between 5 min-6 h timepoints. CORAL tree visualisation, kinase score plots, and STRING-based protein interaction network analyses were used to identify IngM-induced kinase activation patterns and pathway clustering.

Results: IngM induced dose- and time-dependent changes in kinase activities in DFs and KFs. In DFs, IngM induced kinase activation at 5 min, widespread suppression at 30 min, followed by reactivation of Ca^{2+}/calmodulin-dependent protein kinases (CAMKs), cyclin-dependent kinases (CDKs), PKCs, and ribosomal s6 kinases (RSKs) between 1–6 h. In contrast, KFs exhibited sustained activation of mitogen-activated protein kinase (MAPK) and CDK signalling, especially at 0.1 µg/mL IngM, with kinase activation levels exceeding DFs. STRING analyses revealed the kinase families activated, with PKCs centrally positioned within these interactive kinase networks, suggesting their key roles in mediating IngM effects on myofibroblast behaviour.

Conclusions: IngM modulates myofibroblast phenotype and scar matrix composition via the concentration-dependent modulation of PKC-driven signalling cascades, especially at 0.1 µg/mL. Unlike DFs, KFs displayed more persistent kinase signalling, potentially related to their distinct fibrotic genotype/phenotype. Overall, these findings support PKC signalling as a therapeutic instigator of IngM-mediated scar resolution.

P40. PROTOCOLS FOR EXTRACORPOREAL SHOCKWAVE THERAPY ON *EX VIVO* DERMAL SCAR TISSUE

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Background: Every year, 30-72% of burn injuries turn into hypertrophic scars. Hypertrophic scars are characterized by an excessive buildup of connective tissue, which disrupts the skin's structure and function, often causing pruritus, pain, functional impairments, aesthetic concerns, and anxiety. Currently, standard burn care involves pressure application and silicone treatment, but its effectiveness remains insufficient, highlighting the need for improved therapies. Therefore, this study will focus on mechanotherapy, specifically extracorporeal shock wave therapy (ESWT), which turns mechanical stimuli into intracellular signals, promoting scar remodeling. Due to ethical and practical concerns in clinical and animal models, we aim to develop a human *ex vivo* dermal model using cryopreserved healthy and scarred skin to investigate ESWT's effects in a controlled setting.

Methods: During the presentation, I aim to outline the protocol for the planned approach and experimental design for constructing the *ex vivo* model, and hopefully share some preliminary results. Briefly, a pilot study will optimize thawing and tissue preservation protocols by assessing RNA abundance. Furthermore, scarred tissues will undergo controlled mechanical stretching to simulate *in vivo* mechanical forces.

Results: To characterize the model, histological analyses will be used for evaluating tissue morphology and collagen alignment. Once the *ex vivo* model is established, it will be treated with different intensities of ESWT. Next, qPCR will be used to examine fibrotic markers, including α-SMA, TGF-β, and collagen types I and III, to evaluate ESWT's impact on scar remodeling.

Conclusions: Ultimately, this model aims to provide a platform for exploring ESWT's therapeutic potential, addressing ethical concerns, knowledge gaps, and advancing treatment strategies for hypertrophic scars resulting from burn injuries.

P41. SYSTEMIC REVIEW ON WORKING MECHANISMS OF SIGNALING PATHWAYS IN FIBROSIS DURING SHOCKWAVE THERAPY

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Background: Fibrosis is characterized by scarring and hardening of tissues and organs. It can affect every organ system and so could results in organ failure due to the accumulation of extracellular matrix proteins. Previous studies suggest that mechanical forces (such as shockwave therapy, SWT) initiate a process of mechanotransduction and thus could regulate fibrosis. Nevertheless, it is largely unexamined which pathways exactly are involved in the application of SWT and so can regulate fibrosis. The present article seeks to elucidate the underlying effect of SWT on fibrosis.

Methods/Results: Evidence shows that SWT activates macrophage activity, fibroblast activity, collagen amount and orientation and apoptosis which ultimately lead to an adaptation of the inflammation, proliferation, angiogenesis and apoptosis. The included articles reveal that depending on the energy levels and frequency of SWT, other proteins and pathways can be activated. These findings demonstrate that SWT has beneficial effects on fibrosis by influence on the proteins and pathways.

Conclusions: Based on this data that highlights the underlying mechanisms we can make preliminary conclusions about the treatment modalities of SWT in scar formation, such as the energy levels and frequencies that are necessary to prevent or treat fibrotic tissue.

P42. MECHANICAL STRESS – A POTENTIAL CONSIDERATION IN THE DESIGN OF BETTER BIOMATERIALS FOR WOUND REPAIR

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Background: Recent advances in hydrogel and tissue scaffold formulations have provided new opportunities for advanced wound therapies. Despite greater research interest in the field of skin tissue engineering, many studies still focus on material synthesis and characterisations, without considering the physical characteristics of human wounds or the important interactions between wound cells and biomaterials. Here, we present detailed characterisation of human skin mechanical properties from different body sites and age groups and investigate how these properties translate into microenvironment-relevant effects on wound fibroblasts and immune cells.

Methods: Human skin samples were collected with full informed consent, and mechanical properties evaluated using bio-indentation. The same tissue was processed for histological evaluation of dermal matrix composition. Primary human dermal fibroblasts (HDF) in 2- and 3-dimensional spaces were subjected to forces mimicking skin, and cellular morphology/migration tracked using ptychographic imaging. Mouse bone-marrow derived macrophages were cultured on surfaces with different surface rigidity and their cytokines harvested to generate conditioned media for primary mouse dermal fibroblasts.

Results: Standardised indentations demonstrate higher mechanical moduli in pathological compared to healthy human skin, reflected by changes in dermal extracellular matrix composition. Higher constant mechanical stress demonstrated significant negative effects on HDF migrations, while conditioned media from mouse macrophages cultured on substrates with differing elastic moduli, modulated fibroblast proliferation rates and wound healing. These responses were attenuated in cells derived from pathological skin.

Conclusions: The mechanical properties of human skin vary with body site and pathology, and these changes are primarily linked to dermal composition. Primary dermal fibroblasts are functionally responsive to forces delivered by the external environment, and further modified by paracrine signalling from macrophages. Collectively, these studies provide important new insight into the link between skin mechanical properties and wound healing with implications for the design of biomaterials.

P43. THE EFFECT OF ELASTOGENIC COMPOUNDS ON ECM PRODUCTION BY HUMAN DERMAL FIBROBLASTS *IN VITRO*

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Background: Upon skin wounding, one of many challenges is the restoration of a functional extracellular matrix after healing. We previously compiled a list of compounds with diverse chemical structures and mechanisms of action that may promote elastogenesis and found that it was difficult to make a good comparison due the large array of experimental setups used to evaluate their activity.

Methods: In this study, we directly compared a panel of pharmacologically active compounds using primary human dermal fibroblasts from four donors. The tested compounds included retinoic acid (RA), dexamethasone, minoxidil sulphate, transforming growth factor beta 1 (TGF-β1), insulin-like growth factor 1 (IGF-1), copper sulphate ($CuSO_4$), dill extract, magnesium ascorbyl phosphate (an ascorbic acid derivate), heparin, and γ-aminobutyric acid (GABA). The *in vitro* experiments were set up to assess effects on cell viability and extracellular matrix production using Picrosirius Red staining to assess collagen protein deposition, Fastin-Elastin assay to measure elastin content, gene expression analysis for ten relevant genes, immunohistochemical staining for components of the elastin receptor complex (ERC), SDS-PAGE and Western blotting for alpha smooth muscle actin.

Results/Conclusions: Compounds did not show cytotoxicity compared to the control at the same timepoint, although certain compounds influenced the shape of the cells by day 11. Cells exhibited clear signs of proliferation and metabolic activity in time. Under the selected culture conditions, the elastogenic and collagenic impact of minoxidil sulphate, $CuSO_4$, magnesium ascorbyl phosphate, GABA on dermal fibroblasts were negligible. However, dexamethasone, dill extract, IGF-1, heparin, retinoic acid and TGF-β1 emerged as the most promising candidates for dermal therapeutics and skin regeneration strategies.

P45. COLLAGEN AND GLYCOPROTEIN-DERIVED TETRA-PEPTIDES ENHANCE HUMAN KERATINOCYTE PROLIFERATION, RE-EPITHELIALISATION AND WOUND HEALING-RELATED PATHWAYS

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Background: The extracellular matrix (ECM) plays a crucial role during cutaneous wound healing, acting as a structural scaffold and providing signalling cues that promote cell processes such as cell migration and proliferation. Recent studies have demonstrated that ECM-derived peptides termed matrikines, produced through ECM degradation and remodelling, can also influence and modulate cell behaviour in ways not typically observed by native ECM proteins. We have previously shown that two collagen and glycoprotein-derived tetra-peptide matrikines, P1 (Pal-GPKG) and P7 (Pal-LSVD), induced the deposition of dermal fibrillin microfibrils and promoted processes associated with skin homeostasis in photoaged human skin. The treatment of cutaneous non-healing wounds remains a major clinical and financial burden to healthcare systems worldwide, with very limited options available for the treatment of wounds using biologically active molecules. Here, we test the potential of these two peptides to influence epidermal keratinocyte behaviour to promote cutaneous wound healing.

Methods: Primary human epidermal keratinocytes were sourced from three donors (White females aged 51-58, n=3). Peptides modified with a palmitoyl chain, P1 (Pal-GPKG), P7 (Pal-LSVD) and the combination (P1+P7 at a 1:1 ratio) were solubilised in 0.05% w/v DMSO. The ability of the peptides to modulate cell proliferation and migration was assessed using live cell imaging assays and 3D wounded skin models while their effect on the cellular transcriptome was assessed using RNASeq.

Results: Peptides P1, P7 and P1+P7 significantly modulated proliferation for 2 keratinocytes donors, whilst in 3D wounded skin models, P1 and P1+P7 enhanced re-epithelialisation by 7 days post-wounding. Treatment with P1+P7 also significantly modulated the transcriptome of keratinocytes with more than 2000 genes differentially regulated (fold changes >=2) compared to DMSO control

Conclusions: ECM-derived tetra-peptides demonstrate potential for wound healing applications with future work focusing on characterising the bioavailability and mechanistic action of these peptides in cutaneous wound healing.

P46. INVESTIGATING THE EFFECTS OF AN EXPANDABLE COLLAGEN PLUG TO SEAL THE FOETAL MEMBRANES USING A RABBIT MODEL

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[1]Radboud University Medical Centre, The Netherlands.

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Background: The main drawback of fetoscopic surgery is iatrogenic Preterm Premature Rupture of Membranes (iPPROM), occurring due to inadequate membrane regeneration post-surgery. After fetoscopic surgery, ~30% of patients experience iPPROM, often resulting in preterm birth. This project focuses on sealing the membranes using an expandable collagen plug to reduce the risk of iPPROM. In this study we investigate whether the plug is safe to use *in vivo*. Two different plug conditions were used based on the sterilization method since it may affect plug properties.

Methods: Twelve pregnant New Zealand White rabbits were used to test four conditions; positive control (open defect), negative control (no intervention), gamma irradiated plugs, and supercritical CO_2 sterilized plugs. The plugs were introduced through the exposed uterine wall and into the amniotic cavity on day 23 of gestation. On day 30, the tissue along with the plugs were harvested to perform histology. Samples of amniotic fluid (n=7 foetuses) and foetal lung tissue (n=9 foetuses) were collected to check for signs of infection. Ultrasound examination was used to check the presence of foetal heartbeat on the day of the surgery and the harvest.

Results: The plugs could be easily identified as they appeared still intact and properly positioned in the defect site a week after insertion. H&E-stained sections were comparable to the negative control and did not show major infiltration of immune cells into the plug. The amniotic fluid and lung samples tested negative for presence of colony forming units. Furthermore, the survival rate of the test conditions were comparable to the negative control.

Conclusions: As not all data have been analysed, it is too early to draw a final conclusion. However, the plug did not give rise to a foreign body response or infection in the doe or the foetus.

P48. EVALUATING A NEW TOPICAL MICRODOSING APPROACH IN WOUND HEALING APPLICATIONS

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[1]Joanneum Research, Austria.

[2]Medical University of Graz, Austria.

Background: Treating complex wounds, including burns and chronic lesions, remains a persistent clinical challenge. A current focus is the development of wound dressings capable of delivering therapeutic agents in a targeted manner. Hormones represent a class of compounds with notable potential to enhance wound healing; however, systemic exposure poses risks and should thus be avoided. This study explored a microdosing strategy to assess the localized effects of two hormones, i.e. leptin and estrogen, on wound healing dynamics.

Methods: We employed open flow microperfusion (OFM) technology to load leptin and 17-β-estradiol to bacterial nanocellulose (BNC) dressings which were then applied to superficial wounds in both *ex-vivo* and *in-vivo* porcine models. By collecting dermal interstitial fluid (dISF) and blood samples, we assessed both pharmacokinetics and pharmacodynamics. Hormone-loaded BNC dressings were applied to acute superficial excisional wounds, and healing was monitored over a 6-day period. Tissue and blood samples were analyzed to evaluate substance distribution and localized effects on wound healing indicators, i.e. histological analyses.

Results: Neither leptin or 17-β-estradiol was detected in the blood samples. In dISF, leptin peaked at concentrations ranging from 0.5 to 5 ng/ml depending on the implantation depth of the sampling probes after 6 hours, with a decline noted by 12 hours. In contrast, 17-β-estradiol maintained steady concentrations (0.1–1 ng/ml) between 2 and 24 hours. While wound healing progression did not differ significantly between treatment groups, minor variations in epidermal thickness were observed in wounds treated with 17-β-estradiol compared to controls.

Conclusions: Incorporating microdosed therapeutic agents into established wound dressing materials presents a promising approach to enhance the treatment of burns and chronic wounds. This targeted approach should enable localized therapeutic effects while minimizing systemic risks.

P49. INTRODUCING PEERLOGIC, AN AI-SUPPORTED INTERACTIVE DATABASE FOR ANIMAL-FREE CELL CULTURE PROTOCOLS

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[1]Alliance of Dutch Burn Care, The Netherlands.

[2]Utrecht University, The Netherlands.

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[4]F Moser Consultants, The Netherlands.

[5]Sysmo Software, The Netherlands.

[6]Amsterdam University Medical Centre, The Netherlands.

Background: The majority of *in vitro* experiments rely on the use of Foetal Calf Serum (FCS) and/or Basement Membrane Extracts (BMEs such as Matrigel and Cultrex) in their protocols. The use of FCS and BMEs may limit the physiological relevance of *in vitro* models to human conditions, and their production is associated with significant animal welfare issues. Although the number of animal-free alternatives for FCS and BME is increasing, the substitution and implementation of them remains a challenge for researchers as adapting existing protocols requires thorough validation.

Methods/Results: To aid researchers with the transition to animal-free media, Peerlogic*, a new AI-driven open access database has been developed. Peerlogic allows researchers to upload and share their animal-free protocols (including Materials and Method sections of research papers), where AI will extract the relevant details into Peerlogic. In addition, researchers can search Peerlogic for animal-free protocols, share their experiences, add modifications and comment on the use of specific protocols. A rating system has been implemented to aid users in finding successful protocols and which acknowledges the usefulness of protocols uploaded by the users. Peerlogic is a public, non-profit database that works in concert with the FCS-free database** and BME-free database***.

Conclusions: We invite researchers to actively contribute to Peerlogic by sharing their animal-free protocols, helping to accelerate the shift away from FCS and BME in cell culture and promoting more ethical and physiologically relevant research practices.

*https://peerlogic.org

**https://fcs-free.sites.uu.nl/database/

***https://bme-free.sites.uu.nl/database/

P50. COCULTURE OF MULTIPLE CELL TYPES DERIVED FROM A SINGLE INDUCED PLURIPOTENT STEM CELL LINE TO MODEL TISSUE REPAIR AND REGENERATION

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Background: Currently, complex models for testing implanted materials for potential foreign body response (FBR) typically use either mice or human cells from multiple different sources. Using induced pluripotent stem cell (iPSC) -derived cells enables coculture of multiple relevant cell types, from the same donor, with immune cells to better reflect individual patient responses. Here we present a single novel culture medium which can support three cell types derived from the same iPSC line in coculture.

Methods: Fibroblasts, endothelial cells (ECs) and monocytes were derived from iPSCs using a common custom 'base' medium, and incorporation of lineage-specific growth factors to drive differentiation of cells to the desired cell type. Cocultures of these cell types in the base medium were assessed using flow cytometry for lineage-specific surface markers to determine retention of each population, and the medium adjusted to accommodate each cell type, guided by additions required for differentiation.

Results: Flow cytometry was established and optimised for CD140a, CD31 and CD144 antibodies to distinguish fibroblasts and ECs, and demonstrated that in base medium alone fibroblasts proliferated over time while ECs were lost during coculture. To address this, the mechanism of EC loss was investigated and medium and seeding conditions adjusted to compensate, improving retention of realistic cell type ratios to form the basis of a 3D coculture model. Monocyte responses to relevant materials were compared to polarisation by addition of cytokines to assess their responsiveness.

Conclusions: These findings are a critical step towards building syngeneic, 3D FBR models which mimic the complexity of patient tissues and demonstrate that hIPSC derived cells may deliver important information on tissue response to foreign materials. Currently we are including monocytes into the EC/Fibroblast coculture, assembling a structure that better mimics human tissue than 2D culture, and assessing how the model responds to commonly-implanted materials.

P51. DEVELOPMENT OF AN ANIMAL-ORIGIN-FREE 3D CHRONIC WOUND MODEL

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[1]University of Melbourne, Australia.

[2]Peter Doherty Institute Melbourne, Australia.

Background: The development of therapies for chronic wounds is hindered by the lack of representative chronic wound models. Many *in vitro* models are 2D, limited in their complexity and their use of animal products introduces batch-to-batch variability. Current *in vivo* models, specifically mouse models, although more complex than 2D models, possess a profoundly different skin architecture and wound healing cascade compared to humans, limiting research translatability. This project aims to provide an alternative to mouse models by creating an animal-origin-free 3D chronic wound model, with the replacement of animal-derived products. The key benefits are greater ethical research practice, higher throughput for therapeutic screening and greater experimental control.

Methods: Commercially available animal-origin-free cell culture media was evaluated on N/TERT-1 keratinocytes and human dermal fibroblasts, and compared to animal-product-derived media, with DMEM containing FBS. The optimal media was determined following MTT assays, wound repopulation assays and differentiation studies. Two separate 3D *in vitro* models were developed using synthetic peptide hydrogels: a chronic wound model utilising fibroblasts obtained from chronic venous leg ulcer patients and a healthy model using fibroblasts from healthy donors, with keratinocytes seeded on top of the dermal matrix. Histological analysis of the 3D *in vitro* models was carried out using hematoxylin and eosin and Masson's Trichome staining.

Results: Animal-origin-free media supplemented with $CaCl_2$ had lower variability and a similar response on metabolic activity and wound repopulation as the respective control media. Addition of TGF-β_1 to the animal-free media induced fibroblast differentiation and increased expression of alpha-smooth muscle actin. Staining of the chronic 3D model demonstrated less extracellular matrix than observed in the healthy model equivalent.

Conclusions: Animal-origin-free media is suitable for the co-culturing of keratinocytes and fibroblasts, whilst removing any FBS-associated variability. Developing a representative chronic wound model will increase research translatability for therapeutic assessment.

P52. DEVELOPMENT OF 3D WOUND HEALING MODELS FOR NOVEL THERAPEUTIC ASSESSMENT

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[3]Cellese Inc., USA.

Background: The development of therapies for chronic wounds is hindered by the lack of representative chronic wound models. Many *in vitro* models are 2D, limited in their complexity and their use of animal products introduces batch-to-batch variability. Current *in vivo* models, specifically mouse models, although more complex than 2D models, possess a profoundly different skin architecture and wound healing cascade compared to humans, limiting research translatability. This project aims to provide an alternative to mouse models by creating an animal-origin-free 3D chronic wound model, with the replacement of animal-derived products. The key benefits are greater ethical research practice, higher throughput for therapeutic screening and greater experimental control.

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Results: Animal-origin-free media supplemented with $CaCl_2$ had lower variability and a similar response on metabolic activity and wound repopulation as the respective control media. Addition of TGF-β_1 to the animal-free media induced fibroblast differentiation and increased expression of alpha-smooth muscle actin. Staining of the chronic 3D model demonstrated less extracellular matrix than observed in the healthy model equivalent.

Conclusions: Animal-origin-free media is suitable for the co-culturing of keratinocytes and fibroblasts, whilst removing any FBS-associated variability. Developing a representative chronic wound model will increase research translatability for therapeutic assessment.

P53. DEVELOPMENT OF A NOVEL MICROFLUIDIC, IMMUNE-COMPETENT AND VASCULARISED TISSUE-REPAIR-ON-CHIP PLATFORM

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Background: Tissue repair involves complex interplay between various cell populations. Fibroblasts contribute to regeneration by extracellular matrix deposition, while resident macrophages and recruited circulating monocytes differentiate into either pro- or anti-inflammatory phenotypes, aiding wound clean-up, tissue regeneration and remodelling. Evaluating novel materials for tissue repair requires robust preclinical models that reflect this complexity. At the same time, the urgent need to reduce animal use highlights the importance of physiologically relevant *in vitro* platforms. Here, we aim to develop a novel tissue-repair-on-chip device integrating fibroblasts, endothelial cells and monocytes/macrophages for advanced *in vitro* modelling of tissue repair.

Methods: A polydimethylsiloxane-based (PDMS) chip was adapted from a previous design [1]. The model was initially optimised using conventional cell lines (BJ fibroblasts, THP1 monocytes, and HUVEC endothelial cells). To simulate stromal–immune cell interaction and immune cell migration, fibroblasts were seeded in the tissue chamber within a hydrogel (PeptiMatrix™), while immune cells were circulated in the upper chamber, separated by an endothelial cell-coated polyethylene terephthalate (PET) porous membrane treated with laminin. Cells were cultured for 14 days, and cell viability, migration, and phenotype were evaluated using fluorescent staining.

Results: Fibroblasts embedded in the hydrogel demonstrated optimal growth under both static and microfluidic conditions. The growth of endothelial cells seeded on laminin-treated PET membrane formed a confluent monolayer resembling the vascular barrier. Finally, circulating monocytes maintained high viability within the microfluidic circuit and migrated towards the fibroblasts.

Conclusions: These promising results lay the foundation for further development of this novel tissue-repair-on-chip platform by replacing the cell lines with more physiologically relevant induced pluripotent stem cells (iPSCs). This microfluidic model could pave the way for comprehensive evaluation of regeneration-promoting materials by integrating multiple relevant cell types.

P54. DEVELOPMENT AND VALIDATION OF BLUNT FORCE TRAUMA IN A RECONSTRUCTED HUMAN SKIN MODEL

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Background: Forensic medical injury documentation is critical but often insufficient in criminal investigations. Legal experts have emphasized the need for more comprehensive medical information. Current methods for estimating the age of injuries, particularly bruises, remain limited. (Immuno)histological markers, already applied post mortem, may also be suitable for use in living individuals.. The use of immunohistochemistry to date blunt force trauma in living individuals remains largely unexplored. An *in vitro* skin model of superficial blunt force trauma may offer a platform to identify novel markers for forensic injury dating; however, such a model has not yet been developed and validated. This study aims to characterize and validate blunt force trauma in a three-dimensional reconstructed human skin (RhS) model.

Method: Human skin was modelled *in vitro* using a tissue-engineered RhS system, in which human keratinocytes are cultured on fibroblast populated collagen-based hydrogels. Blunt trauma was induced by the impact of a dropped metal object from a height of 10 cm onto the RhS. Morphological changes were assessed by histological analysis.

Results: Blunt force application resulted in visible trauma within the RhS model. Post-trauma, fragmentation of the dermal matrix was observed. Additionally, a reduction in the cross-sectional area of spinous keratinocytes and an increase in epidermal stratification were noted.

Conclusions: This study developed and validated an *in vitro* model of blunt force trauma, providing a basis for the identification of novel injury dating markers in forensic medical research.

P55. PCR BASED PATHWAY DIAGNOSTICS FOR VENOUS LEG ULCERS; MODELING THE HEALTH ECONOMICS AND OUTCOMES ACROSS THE NHS

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Background: Venous leg ulcers (VLUs) are the most common chronic wound aetiology in the UK, with VLU incidence of 0.4% of the population equating to 270,000 new patients per year with a current patient population of 540,000[1]. Analysis from the THIN and SIGN data sets and KOL interviews, show an average of 50% healing rates for a VLU over 12 months. With the direct cost per patient per annum average of £7,706 totalling 1.2% of NHS annual budgets (2018)[3], greater than 80% of costs are attributed to nursing time and inpatient care. VLU treatment pathways follow Pathway 1 (standard of care, SOC) and if not healed, the patient goes onto to pathway 2 (advanced wound care, AWC). The movement of patient care from Pathway 1 to Pathway 2 is a clinical decision with an average time of 18 months[1,2].

Methods/Results/Conclusions: A basic health economics model for the utilisation of a novel gene signature pathway diagnostic[3] has been developed, that diagnoses a patient as a responder to Pathway 1 (heals under SOC) or a non-responder (does not heal under SOC) and requires Pathway 2 (AWC). This model uses diagnostic implementation at first point presentation, full costings, with no change to current practice; other than the allocation of patients to Pathway 1 SOC or Pathway 2 AWC, within 4 days of first presentation.

References

1 Guest JF, Fuller GW, et al. Cohort study evaluating the burden of wounds to the UK's National Health Service in 2017/2018: update from 2012/2013. BMJ Open 2020; 22:10-12.

2 Phillips CJ, Humphreys I, et al. Cost of managing patients with venous leg ulcers. Int Wound J. 2020;17:1074-1082.

3 Bosanquet DC, Sanders AJ, et al. Development and validation of a gene expression test to identify hard-to-heal chronic venous leg ulcers. Br J Surg. 2019;106: 1035-1042.

P56. A PILOT STUDY TO ASSESS THE FEASIBILITY OF VENOUS LEG ULCER PROGNOSTIC BIOMARKER RESEARCH

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Background: Venous leg ulcers (VLUs) often exhibit delayed healing making them costly to treat and manage for health services. Identification of prognostic biomarkers for delayed healing could lead to more accurate healing time estimation, risk stratification and tailored wound care management. However, many prognosis studies are limited by small sample size, short follow-up period and the use of sample collection methods unsuitable for scale-up. Large prospective cohort studies in community settings are therefore required to improve wound healing prognostic biomarker research. Our aim was to pilot a prospective cohort study to identify prognostic biomarkers for VLU healing from patient wound fluid.

Methods: Dressings were collected over time from VLU patients recruited from NHS community clinics across Greater Manchester via the ComplexWounds@Manchester – Biobank. Wound fluid was extracted from dressings using a newly developed method and potential protein biomarkers including matrix metalloproteases and their inhibitors were analysed by ELISA. Protein abundance changes were correlated with healing times.

Results: Over a one-year period 100 participants were recruited across three sites, 75% of VLUs healed and the median time to healing was 10 weeks. Dressings used varied within and between participants. A total of 45 different dressing types were used across the study and 29% of participants were treated with only a single dressing type while enrolled in the study. There were no significant correlations between individual protein biomarkers in extracted fluid and healing time, however a significant positive correlation was found for total protein (Rrm=0.28).

Conclusions: Findings demonstrate the feasibility of recruitment, follow up and VLU wound fluid sample collection from patients attending community clinics using the proposed methodology. Wound fluid protein biomarker measurements were variable and may be affected by known risk factors and the dressing type used. More in-depth statistical methods are planned which will incorporate adjustment for known risk factors.

P57. A PROSPECTIVE COHORT FEASIBILITY STUDY FOR VENOUS LEG ULCER HEALING

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Background: Venous leg ulcer (VLU) healing can be highly variable, making individual healing times difficult to predict. Therefore, identification of prognostic biomarkers for healing would be valuable for patients and wound care practitioners. Large prospective cohort studies are required to generate high quality evidence, however, most VLUs are treated in the community where research infrastructure is limited. Our objectives were to explore the feasibility of conducting a large cohort study for prognostic biomarker identification in VLU patients in the community.

Methods: We conducted a pilot feasibility study in community VLU clinics via the ComplexWounds@Manchester – Biobank. Feasibility questions explored: recruitment, study participation, baseline data, care delivery and data collection methods. Clinical data, wound measurements, ulcer healing, dressing samples and feasibility factors were collected at regular intervals until study exit.

Results: One hundred participants with VLUs were recruited from three community clinics over 1-year. Recruitment, study participation and baseline data methods were considered feasible; with 99% of eligible participants consenting. Attrition rates were low; 75% of participants achieved complete ulcer healing and 9% unhealed, 4% lost to follow-up and 12% withdrawn from the study. Dressings varied within and between participants with 45 different types of dressings used. Hydrocolloid (31.1%) and fabric (25.5%) dressings were most commonly used across the clinics. Median time to healing was 10 weeks (95% CI: 7-12 weeks) from study recruitment. Management of VLUs were relatively uniform across clinics. Missing clinical data was minimal. Data were used to inform cost-effectiveness analyses.

Conclusions: This study enabled the development of infrastructure facilitating sample and data collection in the community setting and provided important information regarding recruitment rates, care delivery in the community and data collection methods. The outcomes will be vital for planning and conduct of a larger study to investigate complex wound prognostic biomarkers.

MSc STUDENT POSTER PRESENTATION ABSTRACTS

M1. ANTIMICROBIAL AND ANTI-BIOFILM ACTIVITY OF APPLE CIDER VINEGAR ON CHRONIC WOUND PATHOGENS

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Background: Chronic wounds (CW), defined as wounds that fail to heal within a normal timescale, represent a significant clinical and economic burden. Standard therapeutics are often ineffective, prompting interests into alternative naturally derived antimicrobials. This study evaluated the antimicrobial activity of three apple cider vinegars (ACVs).

Methods/Results: Minimum inhibitory concentration (MIC) assays against a panel of CW-relevant bacterial species indicated that the MIC_{90} of ACV was 3.13%, whilst povidone-iodine (commonly used in CW dressings) MIC_{90} was two-fold higher. The MIC_{90} of acidified broth was 25%, suggesting that pH alone is not responsible for the observed antimicrobial activity. Exposure of a CW-representative four-species polymicrobial biofilm model to neat ACV resulted in 50% reduction in biofilm biomass within 30 min. Swimming motility is an important biofilm-associated virulence factor and motility assays revealed that ½ MIC of ACV significantly inhibited the motility of *Escherichia coli* NCTC 13846 ($p<0.001$) and *Pseudomonas aeruginosa* PAO1 ($p=0.036$) at 72 h.

Conclusions: These findings suggest that ACV may not only control established biofilm populations within CW, but also limit initial colonisation and attachment, supporting ACV as a low-cost alternative to conventional antiseptics. Future work will include *in vivo* validation of ACV efficacy and safety using the *Galleria mellonella* infection model.

M2. EVALUATION OF THE ANTI-INFLAMMATORY AND WOUND HEALING PROPERTIES OF NATURAL COMPOUND, FICUSEPTINE

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Background: Tropical ulcers are debilitating bacterial infections common in Papua New Guinea. Deploying healthcare infrastructure to remote and inaccessible rainforest locations is not practical, therefore local plants are the best treatment option. Assessment of the efficacy of plant-based, folk-lore medicines commonly used as topical treatments for tropical ulcer by this indigenous population, may also provide new pharmaceutical approaches for the development of novel therapies for similar impaired wound healing scenarios prevalent in Westernized countries, including non-healing chronic wounds such as venous and diabetic ulcers, especially as bacterial infection and chronic inflammation are both hallmarks of these non-healing wounds. Previous work has demonstrated that plant sap derived from *Ficus septica* possesses potent anti-bacterial and anti-inflammatory properties against neutrophils and pro-inflammatory M1 macrophages leading to enhanced ulcer healing *in vivo*, which are hypothesised to be mediated via the major constituent, the alkaloid ficuseptine. Therefore, this pilot study investigated the immuno-modulatory properties of ficuseptine.

Methods: Human blood will be obtained from the Cardiff University Biobank. Neutrophils and non-polarised macrophages were isolated from whole blood. Neutrophils were stimulated with lipopolysaccharide (LPS, 10 μg/mL from *E. coli*), with or without ficuseptine (ng/mL-μg/mL) over 24 h. Isolated macrophages were stimulated to form pro-inflammatory M1 macrophages with granulocyte-macrophage colony-stimulating factor (GM-CSF, 10 ng/mL), with or without ficuseptine (ng/mL-μg/mL) over 7 days. Neutrophil and M1 macrophage activities will be quantified for pro-inflammatory marker (e.g., IL-1, TNF-α) levels by ELISA.

Results/Conclusions: Ficuseptine was confirmed to exhibit concentration-dependent, immuno-modulatory properties against neutrophils and M1 macrophages. Identification of such properties justifies the folk-lore use of *Ficus septica* sap for the treatment of infected tropical ulcers and highlights its potential as a novel therapeutic agent for the treatment of non-haling, infected chronic wounds in Western societies.

M3. IMPAIRED FIBROBLAST WOUND HEALING RESPONSES ASSOCIATED WITH NON-HEALING CHRONIC SKIN WOUNDS: MECHANISMS AND POTENTIAL FOR THERAPEUTIC INTERVENTION

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Background: Chronic, non-healing skin wounds exert heavy physical and psychological burdens on the patients and impose substantial strain on healthcare systems. At the core of this challenge lies dermal fibroblast dysfunction. Under normal conditions, fibroblasts drive the healing process through extracellular matrix formation, wound contraction and tissue remodelling. However, chronic wound fibroblasts display abnormal proliferation, migration, disrupted signalling and premature senescence, due to persistent inflammation and oxidative stress, prolonging the wound state by inhibiting normal healing progression.

Methods: Through this systematic literature review, this Dissertation explores fibroblast behaviour in acute dermal healing and contrasts it with dysfunction observed in non-healing chronic wounds, together with information on how normal cell signalling pathways are altered in chronic wound fibroblasts. Additionally, this review evaluates how research is aiming to develop new therapies to enhance fibroblast activity in non-healing chronic wounds, with particular attention given to emerging therapies such as epoxytigliane compounds.

Results: The review evidence reveals that chronic wound fibroblasts exhibit disrupted TGF-β and PDGF signalling, senescence-associated secretory pathways overactivation, delayed proliferation, defective migration and deficient ECM synthesis, resulting in delayed healing. Analysis of therapeutic strategies highlights their success in restoring fibroblast proliferation, migration, signalling and ECM deposition. Notably, recent research indicates the abilities of epoxytiglianes to positively influence chronic wound fibroblast wound healing responses, in favour of enhanced wound closure.

Conclusions: This review emphasises fibroblast dysfunction as a key contributor to chronic wound pathology, also identifying potential strategies capable of reinstating fibroblast behaviour and signalling, reactivating fibroblast-mediated repair and spotlights the novel compound EBC-46 as an innovative candidate for future fibroblast-targeted therapies.

M4. DEVELOPMENT OF AN *IN VITRO* CHONDROCYTE-OSTEOCYTE CO-CULTURE MODEL OF THE OSTEOCHONDRAL INTERFACE FOR OSTEOARTHRITIS RESEARCH

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Background: Osteoarthritis (OA) is a degenerative joint disease characterised by synovial inflammation and degradation of the articular cartilage and subchondral bone of diarthrodial joints. It typically arises from age-related degeneration or following traumatic injury. Although *in vitro* models of OA exist, animal models are still more frequently used; however, they don't accurately recapitulate the disease and raise significant ethical concerns. Advancing human cell-based *in vitro* models offers a promising alternative, with the potential to reduce animal usage while supporting the development of more physiologically relevant platforms for early-stage drug discovery and testing.

Methods: An immortalised human MSC line was seeded onto a novel cell culture insert and cultured in lineage-specific differentiation media to produce chondrocytes (5 weeks) and osteocytes (1 week). Following differentiation, cell constructs were combined in a co-culture system and subjected to 5ng/mL Interleukin-6, 40ng/mL Interleukin-6 Soluble Receptor and 10mM Yoda1 treatment for 24 hours. This produced a pro-inflammatory and mechanically stimulated osteoarthritic microenvironment through Piezo1 activation. To evaluate the model's efficacy, cell constructs will be analysed for cytotoxicity (CytoTox 96 assay), sulphated glycosaminoglycans (DMMB assay) and nitric oxide (Griess assay). RT-qPCR is currently being used to assess expression of anabolic and catabolic genes including MMP3, aggrecan (ACAN), type II collagen (COL2A1) and FOS.

Results: Cartilage and bone constructs were successfully grown in this novel culture system with initial observations confirming cell viability, attachment and ECM deposition indicative of lineage-specific differentiation of MSCs. Analyses of cell responses are currently being investigated.

Conclusions: This study represents the first documented generation of osteochondral constructs using this modelling system. While molecular characterisation is currently ongoing, this model holds promise as a physiologically relevant tool for OA-focused drug screening. Future work refine model loading to further enhance its potential as an alternative to *in vivo* animal models in exploratory OA research.

M5. OPTIMISING DISSOLUTION PROTOCOLS FOR COST-EFFECTIVE 3D BIOPRINTED EPIDERMAL MODELS

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Background: To overcome limitations with conventional 2D *in-vitro* skin models, developing 3D systems that mimic human skin is crucial. This project utilises a novel, low-cost LEGO-based bioprinter to deposit cell-laden alginate hydrogel scaffolds. These temporary scaffolds require dissolution with citrate-based buffers to allow cellular integration. Unlike standard static culture, custom 3D-printed perfusion cassettes enable precise media flow for controlled scaffold dissolution, ideal for upholding printed spatial organisation. This study aims to validate Polylactic Acid (PLA) as a suitable material for printed perfusion cassette components, and to optimise the dissolution of alginate scaffolds for maximum cell viability.

Methods: Custom PLA inserts (~7.1 cm^2 culture area) were 3D-printed. First, HCA2 fibroblast proliferation ($2x10^5$ cells/well) on PLA versus standard polystyrene (PS) was assessed over 72 hours. Next, fibroblast growth on PLA was investigated over 48 hours using three 1mM Sodium Citrate media: PBS (Media A), DMEM (Media B), and DMEM + FBS (Media C). Finally, byproduct cytotoxicity from dissolving cell-free alginate scaffolds was evaluated to assess print media *(Alginate + Calcium Carbonate + Acetic Acid + Dissolution Media)* impact on cell viability.

Results: Results indicated comparable fibroblast proliferation on PLA and PS surfaces. At all timepoints, mean cell counts were not statistically different. Subsequent experiments showed that cell viability was significantly influenced by dissolution media type, with Media A causing severe cell loss while Media C maintained good viability. Next step is exploring spatial pattern retention using fluorescently labelled cells.

Conclusions: These findings validate 3D-printed PLA as a suitable substrate for fibroblast culture and identify the dissolution parameters for ensuring cell survival post-printing. This work establishes a framework for the future application of the low-cost bioprinter to fabricate more complex, multi-layered human skin models. By optimising these steps, this research contributes to more accessible and physiologically relevant platforms for dermatological research and testing.

M6. SCALE UP OF ANTI-SCARRING SMALL EXTRACELLULAR VESICLES UTILISING 3D SCAFFOLDS

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Background: Scarring remains a significant clinical burden, particularly following burns. To date, limited effective therapies have been developed. Oral Mucosa Lamina Propria-Progenitor Cells (OMLP-PCs) demonstrate positive soft tissue regenerative characteristics including being immunosuppressive, antimicrobial, anti-fibrotic (anti-scarring) and pro-healing. Small extracellular vesicles (SEVs) derived from OMLP-PCs reproduce many of these biological effects including stimulating dermal fibroblast proliferation and wound repopulation, whilst suppressing myofibroblast formation.

Methods: To enable scalable and cost-effective SEV production for clinical use, this project explores the application of 3D scaffolds as a potential scale up tool. OMLP-PCs will be cultured on either 2D plastic or one of three different 3D-scaffolds; (i) 3D PETG scaffolds (Copner Biotech), (ii) 3D fibroblast populated collagen lattices; (iii) 3D bio-printed scaffolds (Grape S1, Copner Biotech). SEVs will be harvested from conditioned media and characterised by nanoparticle tracking analysis, protein:particle ratio and protein concentration. The wound healing potential of the different SEVs will be assessed using fibroblast proliferation assays, fibroblast scratch wound healing assays and a TGF-β1-driven myofibroblast differentiation assay.

Results/Conclusions: This comparative study evaluates SEV yield, bioactivity and production costs across 2D and 3D conditions. By aligning biophysical and functional data with cost-efficiency metrics, this work aims to identify the optimal platform for SEV biomanufacturing. It is envisaged that these findings will support the development of OMLP-PC-derived SEVs as a next-generation, cell-free anti-scarring therapy, bridging the fields of regenerative medicine and scalable bioprocessing.

M7. DENTAL PULP STEM CELL DERIVED EXOSOMES FOR THE TREATMENT OF RETINAL INJURY

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Background: The retina comprises seven specialised cell types that collaboratively process visual stimuli and transmit signals to the brain via the optic nerve. Retinal ganglion cells (RGCs) are critical to this process, functioning as the final output neurons that relay visual information from the retina to the brain. Damage from diseases, such as glaucoma or optic nerve trauma, can result in irreversible vision loss. Current therapeutic approaches remain insufficient in reversing disease progression, typically targeting the risk factors (lowering intraocular pressure) and not directly protecting the neurons. Dental pulp stem cells (DPSCs) have demonstrated therapeutic potential in models of spinal cord and retinal injury, largely through paracrine-mediated mechanisms involving the secretion of neuroprotective factors. Extracellular vesicles (EVs) are emerging as key carriers of these factors and may serve as delivery vehicles for targeted cellular support. Although mesenchymal stem cell (MSC)-derived EVs have shown promise in modulating disease states, the potential of DPSC-derived EVs remains comparatively underexplored. Preliminary studies suggest that DPSC-EVs can support bone regeneration and neural repair in rat models, potentially offering advantages in accessibility and biocompatibility.

Methods: This study investigates the capacity of DPSC-derived EVs to reduce RGC death using an *in vitro* rat model. Conditioned media were harvested from human DPSCs and dermal fibroblasts (HDFs; control), and EVs were isolated via a standard protocol. EV characterisation and quantification were performed using ZetaView® nanoparticle tracking analysis (NTA). Rat retinas were dissected, and retinal cells (including RGCs) were isolated and cultured before the addition of EV treatment at varying concentrations. After 72 h, cultures were fixed, stained, and assessed for RGC survival.

Results/Conclusions: Preliminary NTA data (n=3/group) indicate that DPSC cultures secrete more EVs than HDFs over 72 h. Ongoing analyses aim to determine whether DPSC-EVs confer neuroprotective effects and enhance RGC survival relative to controls.

M8. CAN AN OPTIMAL DOSE OF EXTRACELLULAR VESICLES BE DELIVERED TO THE EYE LONG-TERM USING A MINIMALLY INVASIVE METHOD?

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Background: Corneal opacity is one of the leading causes of vision impairment globally. Current treatments to prevent scarring are application of amniotic membranes (AM) and/or eyedrops. Despite the clinical effectiveness of AM, it can be unpredictable, increase risk of disease transmission, and has ethical complications. While eye drops are less complex, they are also less effective, with insufficient longevity and drug delivery. Several studies have shown oral mucosal progenitor cells (OMPCs) to be responsible for the enhanced wound healing properties of the oral cavity, an effect that has been exploited to promote corneal regeneration. This research aims to develop an eye drop formulation that can overcome these issues by using a fluid gel loaded with extracellular vesicles (EVs) derived from OMPCs.

Methods: The main aim is to optimise the delivery of the EVs and minimise the frequency of application. This should be achieved through the shear-thinning properties of the gel will allow the drop to solidify when stored, return to liquid when shaken prior to application, and then reform as a solid gel on the eye. Blinking fluidises it, allowing the solution to spread while being absorbed. The fluid gel will be prepared by dissolving low acyl gellan gum (gellan) in water at 90°C; then cooled to 60°C and loaded into an AR-G2 rheometer (TA Instruments Ltd.). The gel will then be cooled at 1°C/min whilst being sheared. The EVs will be added at 60° or 40° (batch dependent). Release profiles will be created to compare the effects of viscosity and formulation protocol on the EV release.

Results/Conclusions: This research is one of the first to look at the use of EVs in corneal healing and has potential applications for further development of topical application for other ocular conditions.

M9. PROLONGED RELEASE OF AMY-101 FROM BONE BINDING LIPOSOMES FOR THE TREATMENT OF PERIODONTAL DISEASE

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Background: Periodontitis is a common chronic inflammatory disease that causes gingival inflammation and alveolar bone resorption. The prevalence of periodontitis is very high, with about 62% of the global population having mild to moderate periodontitis and about 23.6% having severe periodontitis. The complement system, especially the C3 component, is the key driver in this inflammatory process. AMY-101 is a new type of C3 inhibitor. In preclinical studies, it has been proven to effectively reduce inflammation and tissue damage, and early clinical trials have also shown its safety and effectiveness. Treatment however requires weekly injections to sustain therapeutic concentrations. This study attempts to use liposomes made of phosphatidylserine (PS) to encapsulate AMY-101, hoping to prolong its retention time in the periodontal area and achieve slow release.

Methods: This study used thin film hydration and extrusion techniques to prepare 100nm PS liposomes, and dynamic/electrophoretic light scattering to measure their size and charge. The binding of PS-liposomes to hydroxyapatite, compared with neutral phosphatidylcholine (PC) liposomes, was observed using a quartz crystal microbalance and fluorescence microscopy. High-performance liquid chromatography (HPLC) was used to measure the drug encapsulation efficiency and to monitor the sustained release of liposomes in solution and in the bound state. The cytotoxicity of AMY-101 on THP-1 cells was evaluated using a CCK-8 assay and its inflammatory response (IL-1β, TNF-α) was measured via ELISA/qPCR, comparing PS liposomes versus free form.

Results/Conclusions: Experiments are still being undertaken, but based on existing studies, AMY-101 has shown potential to prevent the progression of periodontal disease. However, more effective methods of prolonging delivery are required. Liposomes show potential to bind to bone and are likely to help the drug work better locally, therefore creating a reservoir of AMY-101 for prolonged local release. This method may provide a new strategy for the treatment of periodontal disease.

M10. INVESTIGATING THE HEPATIC LIPID-MODUALTING EFFECTS OF HYDROXYTYROSOL IN A MOUSE MODEL OF NON-ALCOHOLIC FATTY LIVER DISEASE AND ATHEROSCLEROSIS

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Background: Non-alcoholic fatty liver disease (NAFLD) and atherosclerosis are major global causes of death, strongly linked to sedentary lifestyles and unhealthy diets. Current treatments, such as statins, are only partially effective and often have side effects. As a result, nutraceuticals are gaining attention as safer, more holistic alternatives. One such compound is hydroxytyrosol (HT), a polyphenol from olives and olive oil with known anti-inflammatory, antioxidant, and lipid-regulating properties. However, its effects on NAFLD and atherosclerosis remain poorly understood. This study aimed to investigate the impact of HT on liver lipids and fatty acids (FAs) in a murine model predisposed to both conditions.

Methods: Two groups of male LDL receptor-deficient mice, which model atherosclerosis and NAFLD, were fed a high-fat diet (HFD). One group received HT supplementation (10 mg/kg/day; 0.81 mg/kg/day human equivalent), and the other served as an untreated control. Previous studies in this lab demonstrated HT's attenuation of HFD-induced atherosclerosis via histological analysis. Lipids were extracted from liver tissue using a chloroform-methanol method. Thin-layer chromatography separated lipid classes into polar lipids (PLs), triacylglycerols (TAGs), and cholesteryl esters (CE), followed by gas chromatography to analyse FA composition.

Results/Conclusions: HT supplementation did not alter total liver lipid content or FA profiles. No significant changes were observed in the FA composition of lipid classes. Analysis of specific changes in PL, TAG, and CE accumulation is ongoing. Future work may include detailed analysis of individual PLs and plasma lipids to better understand systemic effects. Additionally, whether HT inhibits inflammation and the production of reactive oxygen species (ROS) should be explored. Further studies using female mice, other animal models, and human samples may help reveal sex-specific and translational effects. These approaches support growing interest in nutraceuticals to target shared metabolic and inflammatory pathways in NAFLD and atherosclerosis.

M11. MODULATION OF TUMOR-ASSOCIATED MACROPHAGE POLARIZATION BY STATIC MAGNETIC FIELD IN A SQUAMOUS CELL CARCINOMA MICROENVIRONMENT

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Background: Macrophages play a critical role in shaping the tumor microenvironment through their polarization into either pro-inflammatory M1 or anti-inflammatory M2 phenotypes. While tumour-associated macrophages (TAMs) often exhibit an M2-like phenotype that supports tumour progression, recent evidence suggests that physical stimuli such as magnetic fields may influence macrophage polarization dynamics. This study aimed to investigate whether static or pulsed magnetic field exposure can modulate the polarization state of THP-1-derived macrophages, and how such changes affect their interaction with FaDu human hypopharyngeal carcinoma cells in a co-culture model. Using quantitative assays of polarization markers (e.g., CD86, iNOS, CD206, Arg-1), cytokine expression, and functional readouts of cancer cell proliferation and migration, this work explored the feasibility of leveraging biophysical cues to reprogram macrophage phenotypes *in vitro*. This model may open new opportunities for non-invasive modulation of tumour immunity in head and neck squamous cell carcinomas.

Methods: We established an in vitro tumor-associated macrophage (TAM) model by treating PMA-activated THP-1-derived macrophages (MΦ) with conditioned medium (CM) collected from FaDu cells. To evaluate the effect of static magnetic fields (SMF), MΦ were exposed to CM with or without 0.15 T or 0.3 T SMF for 24 h and 48 h. Supernatants were harvested at each time point for analysis. To investigate the cytokine environment and macrophage polarization state, concentrations of TNF-α (M1 marker) and IL-10 (M2 marker) were measured using ELISA. In parallel, gene expression profiling was performed using the Nanostring platform, and differentially expressed genes were further validated by RT-qPCR. All treatments were performed in biological triplicates, and results were analyzed using appropriate statistical software.

Results/Conclusions: Over the next few months, we will complete the establishment of the TAM model, optimize SMF exposure conditions, and begin evaluating macrophage polarization through cytokine analysis and gene expression profiling.

M12. EXPANDING THE CANTIGEN DATABASE FOR IMPROVED PREDICTION OF T CELL TARGETS IN CANCER

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Background: T cell immunotherapy holds substantial promise to revolutionize cancer treatment. The CANTiGEN pipeline is an existing computational tool designed to predict which peptides, derived from tumour-associated antigens (TAAs), are presented by HLA molecules and recognized by T cell receptors (TCRs), facilitating targeted T cell activation. This research aims to expand CANTiGEN's database and improve prediction accuracy through experimental validation and machine learning to reveal better T cell recognition of cancer and identify novel therapeutic targets. Motivation for research stems from the "multipronged" T cell response observed by Dolton et al in cured metastatic melanoma patients, where individual TCRs recognized multiple peptides, leading to enhanced cancer elimination.

Methods/Results: In this research, RNASeq data from the UCSC Xena Platform, encompassing 10 cancer types and healthy tissue was analysed using an in-house R script to identify differentially expressed genes, representing potential TAAs. Additionally, an up-to-date collection of TAAs was collated by cross-referencing publicly accessible databases against the existing CANTiGEN database. A manual literature review limited to 2022-2025 was also conducted, resulting in ~500 TAA-associated proteins that are currently being scanned by CPLs. Existing combinatorial peptide library (CPL) data scanning for cancer reactive T cells using MAT-LAB is currently being undertaken to identify candidate peptides for wet-lab validation by peptide activity assays and MIP-1β measurement. Supervised ML approaches were used to predict peptide immunogenicity based on experimental readouts and CANTiGEN scores.

Conclusions: It is anticipated that this research will accelerate the discovery of effective cancer immunotherapies.