2024 WHS Abstract Session List Key

Young Investigator Awards
Young Investigator Competition....................................................................................................................I

Concurrent Oral Presentations
Infections/Biofilms 1...................................................................................................................................................................................................................................K1
Fibrosis/Scarring 1....................................................................................................................................................................................................................................K2
Acute Wounds/Angiogenesis......................................................................................................................................................................................................................K3
Bioengineering/Biomaterials....................................................................................................................................................................................................................K4
Chronic Wounds 1.......................................................................................................................................................................................................................................K5
Novel Therapies..........................................................................................................................................................................................................................L1
Biological Dressings and Matrices.................................................................................................................................L2
Clinical Indicators...........................................................................................................................................................................................................L3
Chronic Wounds 2..................................................................................................................................................................................................................................L4
Fibrosis/Scarring 2..........................................................................................................................................................................................................................L4
Inflammation..................................................................................................................................................................................................................P1
Burn and Acute Wounds........................................................................................................................................................................................................P2

Rapid Fire Poster Presentations
Rapid Fire Posters.................................................................................................................................................................................................O

General Posters
All Topics .................................................................................................................................................................................................................................................P

Accepted: 22 February 2024
DOI: 10.1111/wrr.13166
Wound Repair and Regen.
Diabetes induces dysregulation of a spectrum of inflammatory cells which contributes to persistent inflammation and defective tissue repair responses. To explore dysregulation of subsets of monocyte/macrophage lineage, we isolated and pooled Live CD45+CD11b+Ly6G− cells from excisional skin wounds of non-diabetic C57Bl/6 and diabetic db/db mice on day 3, 6, and 10 post-injury and performed scRNAseq analysis using the 10x Chromium platform. Cells from non-diabetic vs diabetic wounds exhibited strikingly different phenotypes throughout the healing process. Although wound cells in non-diabetic mice showed an expected transition from pro-inflammatory to pro-healing phenotypes, cells from diabetic mice did not show this transition. Interestingly, a cluster expressing high levels of Cd209a, Cd74 and other genes associated with antigen presenting cells (APC) was populated primarily by cells from non-diabetic mice with significantly lower contribution from diabetic mice. These data indicate decreased accumulation of APCs in wounds of diabetic mice and were confirmed by flow cytometry analysis using markers for this APC cluster. To determine the origin of these cells, we examined this APC population in skin wounds from CCR2 knock-out (KO) mice which lack circulating monocytes. Accumulation of these APCs was almost completely ablated in wounds of CCR2 KO mice compared to their wild-type counterparts, suggesting these cells originate from circulating monocytes. Importantly, adoptive transfer of bone marrow naïve Ly6C+ monocytes to skin wounds indicated that donor cells gained dendritic cell markers including MHC II, CD11c, CD74 and CD209a after 20-hour incubation in the wound environment, confirming the monocyte origin of these APCs. Finally, to begin to understand the regulation of this APC population, we used the BITFAM model to infer TF activity in these cells during skin wound healing, incorporating both scRNAseq and scATACseq data. Predicted TF activity associated with this APC population included PRDM1, IRF4 and STAT4 activity, which was supported by significantly higher protein levels in these cells compared to other wound monocyte and macrophage populations. Previous studies have demonstrated monocytes have the capacity to differentiate into APCs called monocyte-derived dendritic cells which can adopt multi-functional capabilities during inflammation. We are currently performing experiments to elucidate the function of these cells during wound healing. Together, our data demonstrated the significant reduction of monocyte-derived APC in skin wounds of diabetic mice, which may contribute to impaired healing in these mice.
identifies the pivotal role of WE keratinocytes in directing resolution of inflammation, a process often disrupted in non-healing diabetic wounds.

I.03 | Using Microbial Transcriptional Profiles As A Biomarker For Diabetic Wound Healing

Alex Cheong1, Oluchi Aroh2, Caitlin Sande2, Jessica Irvine1, Anna Nora1, Meghan Brennan1, Lindsay Kalan2
1University of Wisconsin, Madison, WI; 2McMaster University, Hamilton, ON, Canada

Introduction: The diabetic foot ulcer (DFU) microbiome has been associated with healing outcomes and is hypothesized to be a source of biomarkers. Profiling the microbiome by 16S rRNA sequencing is limited by species level classification and functional analysis of these communities, especially over time and in response to treatments. Here we apply metatranscriptomics to profile microbial transcription to identify key metabolic activities of the microbiome and use these to predict clinical outcome.

Methods: 100 patients with Wagner grade 1-3 DFUs were enrolled and matched specimens of debrided tissue and deep ulcer swabs were processed for DNA-based 16S rRNA amplicon sequencing and RNA-based metatranscriptomics. Specimens were collected at clinical visits up to week 12 of enrollment. Comprehensive clinical metadata and wound outcomes were collected for each patient. Data was processed to remove human reads, taxonomically classified, and metabolic pathways reconstructed.

Results: 16S rRNA microbiome composition significantly overlapped between the two specimen types, indicating that both are viable sampling methods. However, the metatranscriptome showed swabs are more diverse for species richness and functional diversity as compared to tissue, suggesting an increased sensitivity of RNA-based methods. We used the ratio of RNA to DNA abundance for each genera as a marker for microbial transcriptional activity. Anaerobes were found to be more transcriptionally active in wounds resulting in an amputation. The summed relative abundance of anaerobic transcripts significantly predicted the likelihood of amputation at the end of study (12 weeks; odds ratio 14.17, 95% CI: 1.14 - 232.95, logistic regression, p<0.05). Notably, anaerobic 16S amplicon sequencing relative abundances were not significantly associated with amputation (logistic regression, p>0.05), supporting the increased sensitivity of RNA-based methods. No individual taxa were associated with this outcome (logistic regression, unadjusted p>0.05), but summed transcript relative abundances of Clostridium, Anaerococcus, Peptoniphilus, and Finegoldia species were sufficient to detect a significant association with amputation (logistic regression, p<0.05), highlighting the importance of Gram-positive anaerobic cocci (GPAC) in this dataset. Anaerobic taxa abundance was not associated with peripheral arterial disease and decreased vascular flow, suggesting that there may be local factors (e.g. biofilms) allowing for anaerobes to persist, and that they may function as a separate indicator for low oxygen microenvironments.

Conclusions: These data support the wound microbiome as a promising clinical biomarker. Further analysis of these datasets will yield testable microbial biomarkers that are translatable into clinical tools to be validated in a prospective cohort in collaboration with the Diabetic Foot Consortium.

I.04 | Perinatal Upregulation Of Ccl11 In Skin Fibroblasts Is Essential For Subcutaneous Adipogenesis And Wound Healing

Rahul Debnath, Zhaoxu Chen, Kang Ko
Dental Medicine, University of Pennsylvania, Philadelphia, PA

Background: Fibroblasts are the most abundant mesenchymal cells in the dermis. Though traditionally known for its role in extracellular matrix synthesis, recent advances have provided insight into unexplored immunomodulatory properties of skin fibroblasts during homeostasis and in diseased conditions. Here, we hypothesized that immuno-regulatory secretome by cutaneous fibroblasts is age-dependent and tested if CCL11, a chemokine expressed largely by fibroblasts, is necessary for skin tissue homeostasis that may affect the wound healing process.

Materials and methods: Publicly available scRNA-seq datasets (GSE189210, GSE183031, GSE172226) were pooled, and fibroblast subsets were categorized as neonatal (PO) or adolescent (P22 and P28) for downstream analysis using Seurat package. Among fibroblast transriptome, CCL11 upregulation was most notable, which was validated using FACS followed by RT-qPCR and RNAscope on WT C57BL/6N mice. To determine biological significance, CCL11null mice were examined, and skin specimens were compared to P15 and P28 of age. In a series of animal studies, wound healing was examined in WT and CCL11null mice by histologic analysis and flow cytometry. Autogenous fat grafting in CCL11null mice and CCL11 blocking by neutralizing antibody were examined for healing parameters. Animal studies were carried out with N=4-6 mice each, and statistical significance was determined at p<0.05 by one-way ANOVA and T-test.

Results: Bioinformatics analysis demonstrated that CCL11 transcripts were highly upregulated in the fibroblasts of adolescent mice compared to that of perinatal mice. Further validation showed that CCL11 mRNA was minimally expressed in PDGFRα+ fibroblasts from P3 skin, compared to P15 skin. RNAscope analysis confirmed the age-dependent expression of CCL11, which was largely localized to the dermal layer. CCL11 knockout mice showed a significant reduction in subcutaneous adipose layer width compared to wildtype littermates at P28, suggesting a role for adipogenesis in developing skin. Full-thickness wounds in CCL11null mice had fewer aSMA+ myofibroblasts and delayed re-epithelization compared to WT mice. Fat autografting prior to wound induction in CCL11null mice rescued the healing parameters compared to sham control groups, whereas CCL11 neutralization in WT mice did not have an impact on the wound healing process, indicating that wound healing deficit is dependent on intact adipose tissues and not CCL11.
**Conclusion:** Our study demonstrates that early-life production of CCL11 by dermal fibroblasts is essential for normal adipogenesis, and in turn, cutaneous injury response. The study highlights an important role of fibroblast-derived cytokine production that may unveil pathologic mechanisms behind dysregulated adipogenesis and impaired wound healing.

**I.05 | Host-Biofilm Interaction Derived Oxylipin Impairs Diabetic Foot Ulcer Healing by Targeting T-Cell Immune Checkpoint Pathway**

Sunil Kumar1, Miguel Jorge1, Imran Khan1, Ethan Rinee1, Bryce Hockman1, Kaitlyn Depinet2, Beth Altenburger2, Jaimee Hann2, Gregory Westin1, Mithun Sinha1  
1Surgery, Indiana University, Indianapolis, IN; 2Comprehensive Wound Center, Indiana University Health, Indianapolis, IN

**Background:** Diabetic foot ulcers (DFU) are a leading cause of lower limb amputations. Current DFU treatments rely on pharmacological drugs and standard of care which often results in poor prognosis. The role of bacterial biofilm and its interaction with the host immune system in the context of wound healing is poorly understood.

**Purpose:** This study delves into the connection between DFU and bacterial biofilms, exploring the role of biofilm-derived oxylipins, particularly 10-hydroxy-octadecenoic acid (10S-HOME) and 9-hydroxy-octadecadienoic acid (9S-HODE) in immune cell impairment. Oxylipins have been reported to be immunomodulatory and capable of polarizing CD4+ T cells. **Methods:** To test our hypothesis, we collected wound samples (N=27) from consenting subjects under a protocol approved by IRB. The samples underwent biofilm analysis using scanning electron microscopy (SEM) and 16S rRNA next-generation sequencing (NGS). Metabolite profiling of 10S-HOME and 9S-HODE was conducted via liquid chromatography-mass spectrometry (LC-MS/MS) using deuterated analogs of 10S-HOME and 9S-HODE as internal standard. T-cells from a healthy donor were studied for their immunological role and correlated with human keratinocyte-derived cell line mimicking wound under oxylipin-challenged conditions. Quantitative gene expression and protein abundance were performed using RT-qPCR, ELISA, and dot-blot techniques.

**Results:** Our data from a cohort of 27 patient specimens (wound 17 and control 10) showed a discernible augmentation in bacterial biofilm abundance in the wound samples relative to the control sample. NGS unveiled the presence of microorganisms, specifically Finegoldia magna, Pseudomonas aeruginosa, Staphylococcus aureus, and Anaerococcus vaginalis within the wound tissue. Moreover, oxylipin profiling revealed the upregulation of 10S-HOME and 9S-HODE in the wound tissue compared to normal unwounded skin. Treatment with 10S-HOME induced a discernible retardation in human keratinocyte proliferation compared to sham control (n=10, p<0.0003). Additionally, dysregulation in the expression of the immune checkpoint gene CTLA4 was observed after treatment with the oxylipins 10S-HOME (n=7) and 9S-HODE (n=7) in CD4+T-cells derived from healthy donors.

**Conclusion:** These findings suggest that host-pathogen interaction mediated via biofilm-derived oxylipins may interfere with the healing outcomes of DFU. This alteration seems to be mediated through the regulation of intracellular immune checkpoint genes. Therefore, CTLA4 and other intracellular checkpoints may hold promising candidates in controlling DFUs by targeting specific immune cell subsets. In addition, other checkpoint genes like PD-1 and CISH are under investigation. Further research and verification are essential to establish the potential of this approach in DFU management.

**I.06 | Shell Or The Cargo? Significance of Keratinocyte-Derived Exosomes Surface Molecules In Tissue Repair**

Anu Sharma2, Anita Yadav1, David Clemmer2, Sashwat Roy1, Chandan K. Sen1, Subhadip Ghatak1  
1Surgery, McGowan Institute for Regenerative Medicine, Pittsburgh, PA; 2Chemistry, Indiana University, Bloomington, Indianapolis, IN

**Background** – Exosomes, endocyotically originated extracellular vesicles, play a pivotal role in cellular communication, encapsulating a myriad of biomolecules like DNA, proteins, and metabolites. This study posits that an in-depth analysis of the unique surface composition of exosomal is critical to unravel the intricacies of wound healing.

**Methods** – Keratinocyte-derived exosomes were genetically labeled with GFP-reporter (Exo\textsubscript{\textit{k-GFP}}) using tissue nanotransfection (TNT). Exo\textsubscript{\textit{k-GFP}} were isolated from dorsal murine skin and wound-edge tissue by affinity selection using magnetic beads. Surface N-glycans of Exo\textsubscript{\textit{k-GFP}} were also characterized. Wound-edge keratinocyte-derived exosome uptake was blocked in mice by generating “eat me not” Exo\textsubscript{\textit{k-GFP-RFP}} using a KRT-14 promoter-driven tetraspanins plasmid connected via IRES element with “eat me not”-CD47 sequence with in-frame GFP and RFP reporter (Exo\textsubscript{\textit{k-GFP-RFP}}). Keratinocyte-targeted nanocarriers (TLN\textsubscript{k}) were designed using pH-responsive lipid components with keratinocyte-targeting peptide sequence ASKAIQVFLLAG and loaded with siRNA of hnRNP to selectively inhibit cargo packaging within Exo\textsubscript{\textit{k-GFP}}. The isolated exosomes was characterized as per MISEV 2018 guidelines and by flow cytometry. Additionally, the surface of these exosomes was studied by various spectroscopic techniques such as Raman and FTIR measurements. Functional wound closure was evaluated using analytical histology and Transepidermal Water Loss (TEWL).

**Results** – Our results demonstrate that both compromising cargo packaging within Exo\textsubscript{\textit{k-GFP}} without compromising uptake by blood borne wound macrophages and inhibiting Exo\textsubscript{\textit{k-GFP}} uptake results in the persistence of proinflammatory macrophages at the wound site at day 12 (n=6, n=6). The macrophages exhibited high expression of iNOS (n=4, p<0.001; n=5, p<0.001). However, although no significant difference in re-epithelialization was observed compromising cargo packaging, “eat me not” Exo\textsubscript{\textit{k-GFP-RFP}} significantly compromised wound reepithelialization (n=6, p<0.001). Surface N-glycan analysis
demonstrated a high abundance of mannose on ExoH-GFP-RFP. Raman and FTIR spectroscopic analyses demonstrated spectral differences across the protein, lipids, and nucleic acid domains.

Conclusion - The findings from this research highlight the critical role of exosomal surface molecules in various physiological and pathological conditions. Expanding research on exosomal surfaces is essential for a deeper understanding of the complex healing processes at sites of injury.

1.07 | Structural Equation Model To Quantify Importance Of Patient Factors And Wound Microbiome On Healing

Jacob Ancira³, Rebecca Gabriliska¹, Craig Tipton¹, Clint Miller², Zachary Stickley³, Khalid Omerí, Joseph Wolcott², Todd D. Little³, Caleb Phillips⁴

¹Department of Surgery, Texas Tech University Health Sciences Center, Lubbock, TX; ²Southwest Regional Wound Care Center, Lubbock, TX; ³Department of Educational Psychology and Leadership, Texas Tech University, Lubbock, TX; ⁴Department of Biological Sciences, Texas Tech University, Lubbock, TX

Background: The contribution of microbiome and host factors to driving chronicity and rate of healing in wounds is widely appreciated. However, there is currently little ability to account for the many variables and dynamics influencing differences in healing. Here, with the goal of developing a predictive framework, a novel structural equation modelling (SEM) approach was employed to model the chronic wound environment in relation to healing.

Methods: The dataset consisted of 565 chronic wound microbiomes detected at initial patient visit using 16S sequencing paired with patient medical information. A novel pre-modeling parcel optimization routine was developed to transform the microbiome species table into two latent variables that related to healing time either positively or negatively. These latent constructs in addition to specific species correlating with healing, and patient/wound data were evaluated for model fit in the SEM using backward selection and delta chi-squared testing.

Results: A microbiome latent construct significantly associated with improved healing was validated, and the final SEM included this latent construct plus three species associated with diminished healing (Anaerococcus vaginalis, Fingolagia magna, Pseudomonas aeruginosa), as well as smoking, wound volume, slough, exudate, edema, percent granulation, and wound type. This model explained 49% of variation in healing time with the microbiome contributing the largest proportion of variance explained. Percent granulation and wound volume accounted for the second and third most variance explained. Species that formed the latent construct tended to correlate with each other less than did the remaining species (p < 0.001), potentially reflecting that species associated with faster healing act individually rather than synergistically. Also, species that formed the latent construct that was associated with faster healing also included species that are routinely treated with biofilm-based wound care.

Conclusions: This study provides a novel pre-modeling approach allowing the integration of microbiome data into SEM. The final model validated the importance of many variables on differences in healing time, with wound microbiome species being the most important. The importance of microbes in this model advocates for the efficacy of guiding treatment based on results of DNA sequencing-based microbiota profiling.

1.08 | The Histone Methyltransferase Whsc1 Regulates TGFβ-Driven Macrophage To Myofibroblast Transition During Wound Healing

Kevin Mangum¹, Amrita Joshi¹, Sonya Wolf¹, Jadie Moon¹, Andrea Obi¹, Beth Moore², Frank Davis¹, Katherine Gallagher¹

¹Vascular Surgery, University of Michigan, Ann Arbor, MI; ²Department of Immunology and Microbiology, University of Michigan, Ann Arbor, MI

Introduction: Diabetes contributes to significant morbidity and mortality in the United States, and one of the major features includes poor wound healing, which leads to high rates of amputation due to ineffective treatment options. In diabetes, macrophages exhibit a pro-inflammatory phenotype that prevents normal wound healing. During normal tissue repair, macrophages undergo transition to myofibroblasts, which aid in late wound repair by promoting wound contraction and closure. Our lab and others have shown that macrophages express key fibrotic genes (e.g., SMA, Col1a1, Col3a1), which increase throughout wound healing. However, this fibrotic gene program is disrupted in diabetic macrophages, and fibroblast numbers are reduced in diabetic wounds. Here, we identify Whsc1 as a TGFβ-dependent histone methyltransferase that is essential for macrophage to myofibroblast transition (MMT), and we show that Whsc1 is disrupted in diabetic macrophages.

Methods: The goal of this study was to identify TGFβ-dependent transcription mechanisms that regulate macrophage phenotype during wound healing. Bone marrow derived macrophages (BMDMs) and wound macrophages (CD11b⁺CD3⁻CD19⁻NK1.1⁻Ly6G⁻) were isolated for experiments from a diet-induced obese (DIO) diabetic mouse model and compared with those from normal diet (ND) mice. We performed an epigenetic superarray to identify key chromatin modifying enzymes in macrophages from murine wounds treated ex vivo with TGFβ. siRNA knockdown of Whsc1 and NFκB p65 (RelA) and downstream chromatin immunoprecipitation (ChIP) and qPCR were also used.

Results: Whsc1 was significantly decreased in DIO macrophages with TGFβ treatment compared to ND macrophages (p<0.05). siRNA knockdown of Whsc1 in BMDMs decreased TGFβ-dependent expression of fibrotic (SMA, Col1a1, Col3a1) genes (p<0.05). In ChIP experiments, Whsc1 and its mark H3K36me2 were enriched at fibrotic promoters in ND macrophages in response to TGFβ but decreased in DIO BMDMs. The Whsc1 inhibitor LEM-14 significantly increased wound size and reduced fibrotic gene expression in BMDMs. Interestingly, the pro-inflammatory transcription factor RelA bound more
strongly to Whsc1 in DIO BMDMs. Finally, siRNA knockdown of RelA reversed the dysregulated effect of TGFβ on fibrotic gene expression in DIO BMDMs by increasing Whsc1 enrichment at fibrotic promoters.

**Conclusion:** The current study delineates the TGFβ-Whsc1 signaling axis, which is altered in the diabetic macrophage, thereby leading to defective MMT. Specifically, we show that in ND macrophages Whsc1 controls expression of the fibrotic gene program; however, in DIO macrophages RelA inhibits Whsc1 enrichment at fibrotic gene promoters. Future studies will test whether macrophage-specific targeting of the Whsc1-RelA interaction improves diabetic wound healing in vivo.

**1.09 | Soluble Cd83 Improves Wound Healing And Promotes The Resolution Of Chronic Inflammation In A 3d Wound Healing Model**

Christian Hollard1,2, K. Peckert-Maier1, C. Erfurt-Berge2, A. Steinkasserer1, D. Royzman1

1Department of Immune Modulation, Universitätsklinikum Erlangen, Friedrich-Alexander Universität Erlangen-Nürnberg, Erlangen, Germany, 2Department of Dermatology, Universitätsklinikum Erlangen, Erlangen, Germany

Macrophages (Mϕ) play a pivotal role in orchestrating the wound healing response after skin injury. In the early post-injury phase, pro-inflammatory Mϕ accumulate and sterilize the wound site. Subsequently, these Mϕ undergo a conversion into a pro-regenerative phenotype, initiating the proliferative phase by releasing essential anti-inflammatory cytokines and growth factors. However, when the transition from pro- to anti-inflammatory Mϕ is impaired, the resolution of inflammatory processes is disturbed, thus contributing to the development of chronic wounds. Currently, clinical options for the treatment of chronic wounds are limited and characterized by time-consuming, labor-intensive, and costly procedures. Consequently, there is an urgent need for the identification and development of novel therapeutic agents. In a recently published study, we demonstrated that soluble CD83 (sCD83) enhances and accelerates skin wound healing in mice by inducing pro-resolving Mϕ. Therefore, the focus of this study is to explore the therapeutic potential of sCD83 in the human system using a translational 3D skin wound healing model.

To investigate the effects of sCD83, we generated a 3D wound model using skin equivalents consisting of human keratinocytes, fibroblasts, and Mϕ. Wound infliction was mimicked using a 4 mm biopsy punch. Skin equivalents were treated with either sCD83 (25 μg/ml) or an equivalent volume of PBS as a control. 3D skin equivalents were analyzed by histology and flow cytometry 2 and 4 days after wounding. In addition, the cytokine composition in the supernatants was determined using cytometric bead arrays. In further experiments, chronic wound exudate from patients with venous or arterial/ischemic ulcers was added to the culture medium in order to simulate an inflammatory environment. Under these conditions, the regulatory mechanisms induced by sCD83 under non-healing conditions were analyzed.

The presence of sCD83 resulted in a significantly accelerated wound healing, characterized by an increased influx of cells into the wound area. This effect is mediated by the induction of a pro-regenerative Mϕ phenotype in 3D skin equivalents, as indicated by increased CD163 and decreased CD204 expression levels. Moreover, sCD83 modulates the expression of specific growth factors (increased levels of VEGF, TGF-α, PDGF) and cytokines (increased levels of IL-4, IL-33, decreased levels of IL-1β, IL-23) in wounded 3D skin equivalents. In conclusion, we demonstrate that sCD83 improves wound healing conditions in a 3D wound model, which is a further step towards establishing sCD83 as a therapeutic agent for the treatment of hard-to-heal wounds.

**WHS SESSION K: CONCURRENT ORAL ABSTRACTS I**

**K1: Infections/Biofilms 1**

**K1.01 | What is Slough? Defining the Proteomic And Microbial Composition Of Wound Slough And Its Implications For Wound Healing**

Elizabeth Townsend1, Alex Cheong1, Michael Radzietza2, Blaine Fritz3, Matthew Malone2, Thomas Bjaernsholtt2, Karen Ousey4, Terry Swanson5, Gregory S. Schultz6, Angela Gibson7, Lindsay Kalan1

1University of Wisconsin, Madison, WI; 2Western Sydney University, Penrith, New South Wales, Australia; 3University of Copenhagen, Copenhagen, Denmark; 4International Wound Infection Institute, London, United Kingdom

**Intro:** Slough is a well-known feature of chronic wounds. However, the tissue and microbial composition of wound slough are not well defined. Clinically, it’s difficult to define what is normal slough and identify wounds likely to heal versus deteriorate.

**Aims:** To determine the proteomic and microbiologic components of slough and their associations with wound healing.

**Methods:** 23 subjects with chronic wounds and visible slough were enrolled. Etiologies included venous stasis ulcers, post-surgical site infections, and pressure ulcers. Patient co-morbidities and wound healing outcome 3-months post sample collection were recorded. Debrided slough was analyzed microscopically, through untargeted proteomics, and high-throughput bacterial 16S-rRNA gene sequencing.

**Results:** Wound age ranged from 6 weeks to 15 years. Microscopic imaging revealed slough to be amorphous in structure. 16S-profiling found slough microbial communities associate with wound etiology and location on the body (both PERMANOVA p<0.01). Across all subjects, slough predominantly consisted of proteins involved in skin structure and formation, blood-clot formation, and immune processes. To predict variables associated with wound healing, protein, microbial, and clinical datasets were integrated into a supervised partial least
squares-discriminant analysis. This analysis found wounds that healed 3-months after sample collection were enriched for proteins involved in skin barrier development (eg cornifin B) and negative regulation of immune responses (eg cystatin F). Differential enrichment of these proteins in healed wounds compared to those that deteriorated was confirmed via DEqMS (all absolute Log2(fold change)>1.5 and p<0.01). Wounds that deteriorated over time started off with a higher baseline Bates-Jensen Wound Assessment score and were enriched for anerobic bacteria (eg Fingolda & Peptoniphilus) and chronic inflammatory proteins (eg activator protein-1, vasodilator-stimulated phosphoprotein, & complement factor H; all absolute Log2(fold change)>1.5 and p<0.01).

Discussion: Slough is an underutilized reservoir for potential biomarkers of wound healing. This is the first study to integrate clinical, microbiome, and proteomic data to systematically characterize wound slough and integrate it into a single assessment to predict wound healing outcome. Collectively, our findings underscore how slough components can help identify wounds at risk of continued impaired healing. Future studies aim to explore these predicted biomarkers in a larger cohort. Development of a comprehensive patient-centered assessment will lead to more effective identification of patients’ wounds that may benefit from triage into specialty care and ultimately reduce the healthcare, financial, and personal burden of living with a chronic wound.

K1.02 | Analysis Of 9,241 Wound Specimens Reveals Six Major Microbiome Community Types And Meteorological Associations

Craig Tipton1, Rebecca Gabrilska2, Jacob Ancira3, Courtney Jarvis3, Lars Koenig3, Karin Ardon-Dryer4, Kendra Rumbaugh2, Caleb Phillips1
1Biological Sciences, Texas Tech University, Lubbock, TX; 2Department of Surgery, Texas Tech University Health Sciences Center, Lubbock, TX; 3MicroGen DX, Lubbock, TX; 4Atmospheric Science Group, Texas Tech University, Lubbock, TX

Background: Colonizing microbiota are known contributors to chronic wound persistence. Though differential microbes likely relate to wound management success, there is limited understanding of the major types of microbial communities that are encountered. Prior work in the skin microbiome has shown that geographic and seasonal meteorological variation relates to differences in external microbiota, however it is unknown whether that link translates to a chronic infected wound.

Methods: We set out to describe the microbial composition of the wound microbiome by retrospective analysis of 9,241 specimens submitted from clinics in 43 states for microbial profiling that included matched qPCR, 16S rRNA and ITS gene sequencing. Historical sequence and qPCR data were obtained from MicroGen DX, a CAP/CLIA diagnostic sequencing provider, as matched to patient samples submitted under relevant wound test service between 2018 and 2020. Community state types (CSTs) were identified using the Dirichlet multinomial method. The CSTs were modeled for association to meteorological variables by multinomial logistic regression.

Results: Six CSTs were identified based on bacterial profiles that significantly related to differences in qPCR-estimated bacterial load, alpha diversity, fungal positivity, and bacterial morphotype (e.g., anaerobes). CSTs were unequally distributed across demographic factors such as sex, age, and wound location (p<0.05). For example, men had a 3.6-14.5% increased prevalence of CSTs with high load and anaerobic content (CST3/4) versus a 5.5-18.2% decreased prevalence of CSTs characterized by low bacterial load (CST2/6). The probability of observing CSTs was linked to meteorological variance, particularly temperature and relative humidity. For example, the relative probability of encountering a given CST ranged from -243% to +61% depending on local weather patterns. The most climate sensitive CSTs were characterized by high anaerobic content and load, or low bacterial load not suggestive of infection.

Conclusions: The CSTs identified provide an intuitive description of the types of microbes commonly found and are suggestive of microbial profiles that may warrant different therapeutic approaches. These CSTs provide valuable insights into common assemblages of microbes that will appear in a wound infection, though study in a prospective cohort is required to determine whether the CSTs relate to patient outcomes. Lastly, a statistical association linked the likelihood of observing different CSTs with local meteorological variation, providing the first evidence that wound clinics may encounter different types of infectious profiles and that differences in observation are potentially explained by spatiotemporal weather gradients.

K1.03 | A disposable Device For Fast Screening Of Diabetic Foot Ulcer Infection

Jon Senkowski2, Shuxin Li1, Wenjing Hu1, Liping Tang1
1Progenitec, Arlington, TX; 2Texas Health Physician’s group, Arlington, TX

Purpose: The initial identification of infection in DFU mostly depends on the clinical signs and symptoms of local inflammation. The wound specimens are then collected via wound swab or tissue biopsy for microbiological analysis. Unfortunately, many infected wounds present subtle or no signs and symptoms of infection. Thus, there is an urgent need for the development of a complimentary approach or device that can quickly screen and identify suspected infection in DFU. Recent study shows that infected wounds have elevated leukocyte esterase (LE) activities as compared with non-infected wounds by a portable device - Detec® Esterase. Here, we try to answer two questions. Are LE activities elevated in wound fluids isolated from infected DFU wounds compared to those from non-infected wounds? Can Detec® Esterase be used to screen infection of DFU at point-of-care?

Method: Wound dressings from 15 infected DFU wounds and 5 non-infected DFU wounds based on the culture results were isolated for LE activities measurement. Dressings from 20 DFU patients (14 infected and 6 non-infected) at the Texas Health Arlington
Memorial Wound Care and Limb Salvage Clinic were tested with DETEC® esterase and the device output was compared with subsequent clinical determination of infection on the same wounds. We evaluated the efficiency of the device through sensitivity, specificity, accuracy, and the increase in post-test risk of infection with positive test result.

RESULTS: Our results show that LE activities are significantly higher in infected DFUs (>0.02794 U/mL) than in non-infected DFUs (0.003902-0.02275 U/mL). By comparing our device output with the clinical determination, our results show that pre-optimized DETEC® Esterase has a high sensitivity (~85.7%) and accuracy (75.0%) for screening infected DFU wounds. Further analyses revealed that the diagnosis accuracy of the device is not influenced by the patients’ age, gender, race, initial wound size, wound location, and sign of infection.

CONCLUSION: Our results support that LE activities are elevated in infected DFU wounds, and LE may be used as a biomarker for DFU infection. DETEC® esterase can effectively and accurately diagnose and/or screen for infections in DFUs.

K1.04  |  Pseudomonas Aeruginosa Activates Lasi/R Quorum Sensing, Selective Antioxidant Enzymes, And Type VI Secretion System During Biofilm Formation And Chronic Wound Initiation

Jane Kim, Brandon Le, Weifeng Gu, Manuela M. Martins-Green
University of California, Riverside, Fullerton, CA

Pseudomonas aeruginosa (PA) is an opportunistic pathogen frequently isolated from cutaneous chronic wounds. Some PA strains are highly virulent, establish strong biofilm and are antibiotic resistant. How PA colonizes chronic wounds with biofilm formation in response to oxidative stress (OS), is still unknown. The purpose of this study is to investigate the changes in gene expression when PA is challenged with high levels of OS, and how it increases virulence, decreases OS and becomes biofilm-forming in chronic wounds. We used a biofilm-forming PA strain isolated from the chronic wounds of our murine model and performed qPCR to obtain gene expression patterns as PA developed biofilm in vitro in the presence of high levels of OS and then verified the findings in vivo. We found that the planktonic bacteria while forming biofilm under OS conditions, overexpressed the Quorum Sensing genes lasI and lasR, rhlI, and rhlR, which are important for the bacteria to communicate with each other to form biofilm. We also found that the antioxidant stress genes sodA, sodB, katA, katB and oxyR important in reducing the OS in the microenvironment for survival, were also overexpressed. In addition, expression of biofilm formation genes such as pelA and pelB and pslA and pslB and virulent genes phzA and phzM for phenazine production were increased. In vivo, we found that the levels of gene expression for quorum sensing, biofilm formation, and virulence were all upregulated during biofilm development. However, of the antioxidant genes, only oxyR, katA and sodB were upregulated. To identify unique genes, we performed a broader transcriptomic analysis of PA using the RNAseq in vivo and identified the activation of novel genes/pathways of the Type VI Secretion Systems involved in PA pathogenicity. Several genes of the core secretion structure, including tssB and tssC, and phospholipase effector proteins, such as tliS, are significantly upregulated. PA is known to activate the Type VI Secretion System to compete with other bacteria in its microenvironment and target mammalian cells for infection. In conclusion, PA survives the harsh, high OS microenvironment present in chronic wounds and colonizes these wounds with biofilm by turning on virulent, biofilm-forming and survival genes. Therefore, effective biofilm removal and or return after debridement may be accomplished by disrupting the lasR system and/or sodA, katA and katB expression and potentially also the Type VI Secretion System. Because it is well known that biofilm in human chronic wounds readily returns after debridement, these findings could have implications for treatment of human chronic wounds to eliminate PA containing biofilm immediately after debridement.

K1.05  |  Detection And Photoablation Of Wound Biofilm Infections With Theranostic Gold-In-Gold Cage Nanoparticles

Maryam Hajfathalian1, Christiaan R. de Vries1, Yuxi C. Dong2, Ahmad Amirshaghahi2, Pallavi Jonnalagadda2, Jessica HSU2, Aimen Zlitni1, David Cormode2, Paul Bollyky1

1Stanford University, Mountain View, CA; 2University of Pennsylvania, Philadelphia, PA

Introduction: Bacterial biofilms colonize wounds and delay healing. These biofilms are difficult to treat with existing clinical therapies due to antimicrobial resistance. Therefore, a critical need exists to effectively diagnose and treat biofilm infections. Here, we present a theranostic agent to image and treat harmful biofilms. We developed gold-in-gold cage nanoparticles (PTNP) with enhanced photothermal (PTT) and photoacoustic imaging (PA) properties that are promising candidates for biofilm detection, treatment, and infectious disease control. We found PTNP can control virulent biofilms and treat infectious disease via activation with near infrared region (NIR) laser with precise spatial control and in a short timeframe. A strong biocidal effect against Staphylococcus aureus (S. aureus) within biofilms was observed, considerably more effective than currently clinically used skin antimicrobials. Therefore, here, we introduce a fast, precise, and unique topical therapeutic method to image and treat costly skin biofilm-associated infections.

Methods: PTNP were synthesized via galvanic replacement reaction. After purification, the collected nanoparticles were coated with dextran-10kDa (DEX) to provide stability in biological media. These structures were characterized using transition electron microscopy (TEM), energy dispersive X-ray spectroscopy (EDS), and UV-visible spectroscopy. The anti-biofilm efficacy of the PTNP was examined in vitro using S. aureus biofilms. The untreated or treated biofilm with PTNP was analyzed using high-resolution confocal fluorescence imaging, while PA imaging of biofilms treated with PTNP was carried out to investigate the theranostic potential of these structures within the
Extracellular Granzyme B Contributes To Delayed Healing Of Cutaneous Leishmaniasis In A Murine Model

Layla Nabai1, Yasaman Kaviani1, Katlyn Richardson1, Alexandre Aubert1, Karen Jung1, Farhad Handjani2, Reza Yaghoobi3, Nicholas Carr2, Hongyan Zhao1, Robert McMaster5, Hongyan Zhao1, Robert McMaster5, Hongyan Zhao1, Robert McMaster5

**Background and Hypothesis:** Cutaneous leishmaniasis (CL) is an infectious disease caused by different species of leishmania parasite, resulting in a variety of chronic skin lesions that leave severe scarring and disfigurement. Granzyme B (GzmB), a serine protease, has been implicated in pathogenesis of CL caused by *Leishmania braziliensis*. Although the cytotoxic role of intracellular GzmB in parasite killing and targeted apoptosis in CL is well known, the contribution of extracellular GzmB to chronic inflammation, tissue damage, and scarring seen in CL is poorly understood. Here, we hypothesized that GzmB is elevated in CL caused by *Leishmania major* (*L. major*) and contributes to impaired healing through the cleavage of cell-cell and cell-basement membrane adhesion proteins.

**Methods:** Paraffin-embedded human skin biopsy specimens with confirmed diagnosis of *L. major* induced CL were subjected to the histological analysis using hematoxylin&eosin, immunohistochemistry (IHC), and immunofluorescent staining for GzmB, cellular markers of different immune cells, and GzmB substrates. Cell-free in vitro cleavage assay was used to identify new GzmB substrates. Twelve wild type and 11 GzmB knockout C57BL/6 mice were inoculated with *L. major* and the clinical course of the disease was documented over 8 weeks. Skin samples were collected for histological analysis at study endpoint.

**Results:** GzmB was highly expressed in human CL lesions compared to normal skin. The expression of E-cadherin, a cell-cell junction protein was significantly reduced in areas with higher number of GzmB expressing cells (n=11, p<0.001). Collagen VII and collagen XVII, were also reduced in areas with accumulation of GzmB+ cells. Cell-free in vitro cleavage assay resulted in identification of two novel substrates of GzmB, desmoglein 4 and annexin A2. Further, IHC staining of human samples showed reduction of desmoglein 4 in samples demonstrating psoriasiform dermatitis, and annexin A2 on endothelial cells of thrombotic blood vessels. In vivo study revealed that while 54% of GzmB KO mice showed no visible lesion/scarring, all WT mice still had clinical lesion/scarring at the study endpoint.

**Conclusion:** Our results show that extracellular GzmB is associated with epidermal changes and vascular thrombosis in *L. major* induced CL and deletion of GzmB significantly reduces the healing time of the lesions in a murine model of CL. Collectively, our findings suggest a prominent role for extracellular GzmB in pathogenesis of skin lesions caused by *L. major*.

Fibrosis/Scarring 1

Circulating Mechanoresponsive Myeloid Cells Contribute To Fibrosis Across Disease States And Organ Systems

Kellen Chen2, Dominic Hess2, Dharshan Sivaraj2, Katharina S. Fischer2, Jagannath Padmanabhan1, Michael Januszyk1, Geoffrey C. Gurtner2

**Background:** Tissue injury activates signaling pathways to recruit different cell types to orchestrate the healing response. Excessive cell activation and recruitment leads to prolonged inflammation and fibrosis with overproduction and accumulation of extracellular matrix (ECM) proteins, leading to dysfunctional scar. In this study, we identify and modulate mechanoresponsive myeloid cells that contribute to fibrosis during both cutaneous hypertrophic scar (HTS) and biomedical implant foreign body response (FBR) formation in humans, mice, and pigs.

**Methods:** We performed single cell RNA sequencing and/or Visium spatial transcriptomics on human and mouse scar and healthy skin tissue, as well as severe and mild FBR tissue. We collected human tissue with approved IRBs. We utilized murine HTS (Arabi 2007, Chen 2024 unpublished), pig wound (Chen 2021, Chen 2022), and murine FBR (Padmanabhan, Chen 2023) models. In each of these, we physically modulated the mechanical signaling environment to increase fibrosis...
and used genetic knockout (KO) and/or pharmacological disruption of mechanotransduction to reduce fibrosis.

**Results:** In humans, we observed that mechanical signaling pathways were distinctly upregulated in severe HTS (unpublished) and FBR (Padmanabhan, Chen 2023), compared to unwounded/benign HTS/FBR. Mechanoresponsive myeloid cells upregulated pathways related to the Rac/Rho and FAK pathways to drive downstream fibrotic pathways. In murine HTS, mechanical strain significantly increased scar formation and inflammatory recruitment (p<0.01), while both KO and pharmacological disruption of myeloid mechanical signaling mitigated scar and restored regenerative Thbs-/Egr1+ myeloid subpopulations. In murine FBR, increasing mechanical forces significantly increased pathological FBR to human levels (p<0.01), while both KO and pharmacological disruption of myeloid subpopulations, leading to skin tissue regeneration (p<0.01), with restoration of collagen architecture and skin mechanical properties (p<0.001) (Chen 2021, Chen 2022).

**Conclusions:** In these studies, we observe that in both humans and animals, mechanosensitive myeloid cells predominantly push the development of pathological fibrosis, which then stimulates fibroblasts to produce excessive amounts of collagen. This previously unappreciated link and reciprocal relationship between mechanosensitive myeloid cells and fibroblasts could be exploited therapeutically in humans, such as using a combined mechano-inhibitor to prevent pathological FBR or severe HTS.

---

**K2.02 | Direct Contact With Mechanically Activated Myofibroblasts Drives Macrophages Into Distinct Transcriptional States**

Li Diao1, Ronen Schuster2, Fereshteh Younesi1, Boris Hinz1

1LTRR, Unity Health Toronto, Toronto, ON, Canada; 2CytoReason, Tel Aviv, Israel

**Background:** Both fibroblasts and macrophages (MΦ) are key in promoting the formation and remodeling of extracellular matrix following organ injury, but aberrant crosstalk can contribute to the development of fibrosis. MΦ provide cytokines like TGF-β1 that stimulate fibroblast activation into contractile myofibroblasts (MF). We have published that MF activation by MΦ-derived TGF-β1 requires spatial proximity and a ‘scar-stiff’ tissue environment. However, little is known how, in turn, MFs control MΦ phenotypes in the context of tissue repair and fibrosis. We hypothesized that mechanically activated MFs control the development of fibrosis. MΦ provide cytokines like TGF-β1 that stimulate fibroblast activation into contractile myofibroblasts (MF). We have previously shown that MF activation by MΦ-derived TGF-β1 requires spatial proximity and a ‘scar-stiff’ tissue environment. However, little is known how, in turn, MFs control MΦ phenotypes in the context of tissue repair and fibrosis. We hypothesized that mechanically activated MFs control distinct MΦ states in contact-dependent signaling processes.

**Methods:** MΦ were obtained by treating mouse bone marrow-derived monocytes with M-CSF in vitro for 5 d. Subcutaneous fibroblasts were isolated from Col1a-GFP reporter mice. To mechanically establish fibroblast and MF populations, fibroblasts were cultured on skin-soft or scar-stiff gelatin-coated silicone substrates for 2 passages, respectively. MΦ were then co-cultured for 3 d with fibroblasts/MFs on the respective substrates in setups that allowed either direct contact or medium-sharing only. Cells obtained from all experimental combinations were separately analyzed using immunofluorescence (IF) confocal microscopy and flow cytometry. Fibroblastic cells and MΦ were flow-sorted for subsequent RNA sequencing, further analyzed for principal components, differentially expressed genes and enrichment of signaling pathways and transcription factors binding motifs.

**Results:** Fibroblasts cultured alone on stiff substrates exhibit MΦ protein and RNA profiles absent from soft-cultured fibroblasts. Substrate stiffness in the chosen range does not affect RNA profiles of MΦ in monoculture. Conversely, co-culture with fibroblastic cells results in significant changes in MΦ transcriptomes, with unique features depending on the activation state of the co-cultured fibroblasts and the ability to form direct contact. Specifically, (1) MΦ in direct but not medium-shared-only co-culture with fibroblastic cells acquire an activated MAPK signaling. (2) Direct contact with fibroblasts results in suppression of stress response to stimuli and inter- and intra-cellular signal transduction. (3) In direct contact with MΦ, MΦ exhibit upregulated pro-fibrotic signaling pathways, mediated by the activation of semi-plexin-GTPase axis and IL-17, NF-kB, and C-type lectin signaling. IF and flow cytometry validate RNA sequencing data. For instance, MΦ exhibit up to 1.6-fold significantly increased expression of CD206 in MΦ co-cultured with MΦ versus fibroblasts. Increased expression of MΦ CD206 is 6-8-fold more pronounced in co-cultures with direct contact.

**Conclusion:** Direct contact with MFs generate a unique MΦ polarization state. The recognition of such a state offers novel therapeutic targets and potential for the prevention and treatment of fibrosis.

---

**K2.03 | The Role of Periostin and Hyaluronan Crosstalk in the Regulation of Wound Fibrosis**

Sonya Keswani, Tanuj J. Prajapati, Hui Li, Ling Yu, Swathi Balaji

Baylor College of Medicine, Houston, TX

Postnatal dermal injury triggers inflammatory and fibrogenic reactions involving feedback signals between ECM mediators that contribute to either wound regeneration or fibrosis. We identified a role for high molecular weight hyaluronan (HMW-HA) in promoting wound regeneration. Upon injury, HA and periostin (POSTN), a matricellular protein, are upregulated. However, HMW-HA expression is not sustained and quickly tapers down in postnatal wounds, while POSTN is sustained. We hypothesize that HA and POSTN crosstalk regulate ECM balance and wound fibrosis.

In vitro, murine dermal fibroblasts (mdf) from C57BL/6J mice were stimulated with TGFβ1 for 24h to promote fibrogenic milieu. HA and POSTN crosstalk was analyzed using gain- and loss-of-function of HA and POSTN signaling. The effect of siRNA knockdown of POSTN on
HA synthesis and HA degradation enzymes was assessed. In vivo, 6mm full-thickness stented wounds in C57BL/6J (B6) mice were studied with +/- lentiviral overexpression of HMW-HA, and the effect on HA and POSTN expression at d7 and d28 was examined along with changes in collagen and ECM architecture. Human dermal fibroblasts (hDF) from patients with low(LS) and high scar(HS) phenotypes clinically stratified with VSS <3 vs. >6 were tested. p values by ANOVA.

TGfβ1 treatment upregulated both HA-synthase (HAS2) that synthesizes HMW-HA and POSTN gene expression in mDF. To determine if POSTN regulates HAS2 in this model, we knockdown POSTN using siRNA, which significantly reduced the expression of HAS2 (p<.05). Furthermore, mDF treated with 50ng/ml POSTN for 48h showed significantly higher mRNA levels of HAS1 and 2 than controls. To further assess the impact of HAS2 on a reciprocal feedback mechanism that regulates POSTN, HAS2-specific siRNA knockdown was done, which caused a significant increase in POSTN mRNA and protein. In vivo, POSTN was upregulated upon wounding. HMW-HA induction further increased POSTN at d7 post-wounding, but reduced its expression compared to PBS wounds at d28 of the remodeling stage which coincided with reduced collagen and improved ECM architecture in HMW-HA induced wounds at d28. This suggests that sustained HMW-HA production could counter POSTN expression. It is cogent that when HAS2 is low, POSTN expression is upregulated, but when HAS2 expression increases, POSTN is inhibited. Treatment of patient skin fibroblasts LS-hDF vs. HS-hDF with POSTN resulted in differential regulation of HAS2 expression, indicating that each subject responded to POSTN differently, which may contribute to the heterogeneity observed in human wound healing.

Our data suggest a mutual HAS2/POSTN interdependence. While injury-induced POSTN is necessary for HAS2 signal induction, the HAS2/HMW-HA then suppresses POSTN to promote wound regeneration. Understanding these basic mechanisms will facilitate improved wound healing and lead to regenerative tissue repair.

**K2.04 | Microfibril Associated Protein 5 And The Regulation Of Skin Scar Formation**

Chen Han1, Heidi Yuan1, Lin Chen1, Timothy J. Koh1, Robert P. Mecham2, Luisa A. DiPietro1

1Center for Wound Healing and Tissue Regeneration, University of Illinois College of Medicine at Chicago, Chicago, IL; 2Department of Cell Biology and Physiology, Washington University School of Medicine, Saint Louis, MO

**Background:** Studies in our lab have shown that Microfibril Associated Protein 5 (MFAP5, or microfibril-associated glycoprotein2/MAGP2) is upregulated in skin wound healing and modulates fibroblast phenotype. MFAP5, a 25 kD extracellular matrix (ECM) glycoprotein, is linked to fibrosis and angiogenesis in cancers and fibrotic diseases. The aim of this study was to use MFAP5 deficient (Mfap5−/−) mice to investigate MFAP5’s role in wound healing and on fibroblast transcriptome and function.

**Materials & Methods:** Full-thickness excisional wounds were made on dorsal skin of Mfap5−/− and C57BL/6J control (Mfap5+/+) mice. External wound closure was assessed, and wound tissue was collected during healing to assess key features of wound repair (N=9-10). Wound angiogenesis and inflammatory cell content were assessed by immunofluorescent staining of CD31 or Ly6G and CD68, respectively. Collagen deposition and maturity were assessed by Masson’s Trichrome and Herovici staining, respectively. To examine how loss of MFAP5 affects fibroblast transcriptome and phenotype, skin fibroblasts were isolated from neonatal Mfap5−/− and Mfap5+/+ mice. RNA-sequencing was performed on Mfap5−/− and Mfap5+/+ fibroblasts (N=6). All significantly downregulated genes (padj<0.05) in Mfap5−/− versus Mfap5+/+ fibroblasts underwent gene ontology enrichment analysis and annotated to biological processes (BP). Cellular migration, contractility, and proliferation were compared between Mfap5−/− and Mfap5+/+ fibroblasts (N=14-20). To examine changes to ECM composition, Mfap5−/− and Mfap5+/+ fibroblasts (N=8) were grown to confluency, treated with ascorbic acid, and then immunocytochemistry stained for ECM proteins.

**Results:** Mfap5−/− mice had significantly reduced rates of skin wound closure and wound angiogenesis (p<0.05). Mfap5+/− mice also had significantly enhanced inflammatory cell content as compared to Mfap5+/− mice (p<0.05). Collagen deposition in Mfap5−/− mice was significantly reduced in normal skin (NS) and at 21 days post-wounding, while only NS of Mfap5−/− mice had significantly altered collagen maturity versus Mfap5+/− mice (p<0.05). RNA-sequencing revealed down-regulated BP related to ECM organization, cellular migration, and proliferation. Mfap5−/− fibroblasts had significantly reduced cellular migration, contractility, proliferation, and COL1A2 deposition (p<0.05).

**Conclusions:** Our results suggest that MFAP5 is important for wound closure and angiogenesis, MFAP5 is also likely involved in the maturation and organization of collagen and may influence skin wound inflammation. Lastly, our in vitro assays demonstrate that MFAP5 is a regulator of fibroblast characteristics important for scar formation.

Funding: R01 GM050875, R35 GM139603, F31 DE028747, F31 AR082287.

**K2.05 | Elucidating the Role of sFRP2 in Modulating Organ Fibrosis**

Delany Bradford1, Pampee Young2, Sarika Saraswati1

1Tennessee State University, Nashville, TN; 2American Red Cross, Nashville, TN

**Background:** Fibrosis is the typical response to injury, which leads to distorted architecture, pathologic signaling and ultimately organ dysfunction. In cardiac tissue specifically, a fibrotic response to injury can lead to a decrease in the heart’s ability to function, which plays a significant role in the pathogenesis of most heart diseases. Secreted
frizzled-related protein (sFRP2) has been identified as a mesenchyme derived factor that augments post-myocardial infarction repair, in part by down-regulating fibrosis. Yet, the molecular mechanism that regulates sFRP2’s effect on fibroblasts in modulating tissue fibrosis is incompletely understood.

**Methods:** We have generated a transgenic mouse model in which we can temporally and spatially regulate the expression of sFRP2 in injury-induced activated FSP1+ fibroblasts. These transgenic mice received bone marrow transplantation (BMT) from C57BI/6 mice to ensure specificity of sFRP2 protein in fibroblasts, since FSP1 is also expressed in hematopoietic cells. sFRP2 expression was induced post-infarct following tamoxifen treatment in mice expressing sFRP2 under FSP1 promoter. These mice were assessed in vivo and in vitro for injury-induced fibrotic responses in two distinct models, heart and skin.

**Results:** Post-injury induction of sFRP2 exerted an anti-fibrotic effect in comparison to control Cre mice in heart as well as skin. sFRP2 overexpression in heart following myocardial infarction resulted in reduced scar size, improved function, and reduced adverse cardiac remodeling. In addition, assessment of the collagen content following excisional wound (skin injury) demonstrated significant reduction in dermal collagen deposition in sFRP2 overexpressing transgenic mice. Significant reduction in Wnt signaling, TGF beta signaling and collagen production was identified in mice overexpressing sFRP2.

**Conclusion:** Post-injury sFRP2 over-expression in mouse FSP1-fibroblasts resulted in reduction in fibrosis in two different organ injury models. Elucidating the mechanism that modulate sFRP2’s antifibrotic role will provide valuable insights on how this protein targets fibrosis and promotes regenerative repair. Identification of sFRP2’s role will also provide a potential use for such regulators as a way to target specific post-injury fibrotic processes such as Wnt and TGF beta signaling and inhibit pathological fibrosis without interfering with normal wound healing.

**K2.06 | Divergent Contributions Of Systemic Immune Cells And Local Fibroblasts To Wound Closure And Fibrosis**

Andrew Hostler, William Hahn, Jenne Stensland, Katharina Fischer, Maria Gracia Mora Pinos, Jared S. Holley, Abdelrahman Alsharif, Jonathan P. Yasmeh, Fidel Saenz, Autumn Lester, Hudson C. Kussie, Eamonn McKenna, Maia Granoski, Amelia B. Knochel, maisam Jafri, Jose Vasquez, Geoffrey C. Gurtner, Kellen Chen

**Surgery, University of Arizona College of Medicine -Tucson, Tucson, AZ**

**Background:** Tissue repair is a dynamic process requiring various cellular populations and non-cellular elements coalescing to restore functional tissue integrity. Chronological aging has been associated with impaired physiological wound healing via diminished inflammatory and fibroblast cells. While we know that impaired immune cells and dysfunctional fibroblasts impede wound healing within aged populations, limited evidence exists isolating the importance of one cell type over the other. To further characterize the age-related cellular mechanisms of wound healing, we developed a novel skin graft murine model to isolate the effects of impaired, aged systemic immune cells versus dysfunctional local fibroblasts.

**Methods:** We established a novel heterochronic full-thickness skin graft (FTSG) murine model utilizing both 8-week (young) and 80-week (old) C57BL/6J mice. We harvested FTSGs to transplant old skin (OS) onto the dorsum of young mice (Y+OS). Conversely, the harvested young skin (YS) was transplanted onto the backs of old mice (O+YS). Young mice receiving young mouse skin transplants were used as controls (CO). Additionally, unwounded skin also analyzed (US).

Upon FTSG integration (40 days), we utilized our splinted excisional wounding model to create two cutaneous, dorsal wounds at the graft region. Gross wound size was measured to quantify wound closure rate, and healed wound tissue was explanted at postoperative day (POD) 20. Hematoxylin & Eosin (H&E), Masson’s Trichrome, and Picrosirius Red staining was performed to access epidermal and dermal thickness, dermal collagen density, and dermal integrity.

**Results:** Control and Y+OS mice healed at around POD ~15.5 while O+YS took up to POD 20 (p=0.0001). The rate of wound closure was significantly decreased in O+YS mice compared to Y+OS mice on PODs 6 (p=0.007), 8 (p=0.005), 10 (p=0.003), 12 (p=0.0001), 14 (p=0.002), 16 (p=0.02), and 18 (p=0.03). Subsequently, the rate of wound closure was significantly decreased in O+YS mice compared to control mice on POD10 (p=0.005), 12 (p=0.0001), 14 (p=0.0002), 16 (p=0.006), and 18 (p=0.02). Software CT-FIRE and CurveAlign were used to assess collagen fiber structure. We observed that CO and O+YS significantly increased collagen density (p<0.001) compared to US while Y+OS did not demonstrate an increase.

**Conclusions:** Our results demonstrate an intriguing divergence of immune and fibroblast contributions during the process of wound healing and fibrosis. Regardless of the transplanted skin, young mice demonstrate accelerated wound closure, thus showing that the young circulating immune cells primarily contribute to the wound healing process as opposed to local cells. In contrast, regardless of the age of the mouse, mice receiving young skin demonstrated more fibrotic skin after injury, demonstrating that the old local fibroblasts dominate the fibrotic response over the systemic contributions.

**K3: Acute Wounds/Angiogenesis**

**K3.01 | Single-Cell RNA-Sequencing Identifies Novel Molecular Targets of Endothelial MicroRNA-200b in the Diabetic Ischemic Wound**

Kanhaiya Singh, Manishekhar Kumar, Sujit K. Mohanty, Savita Khanna, Sashwati Roy, Chandan K. Sen

**McGowan Institute for Regenerative Medicine, Department of Surgery, University of Pittsburgh, Pittsburgh, PA**

Injury-induced transient downregulation of endothelial miR-200b is required to jump-start wound angiogenesis, but the underlying
mechanisms remain unclear. Single cell RNA sequencing was performed on ~15000 human endothelial cells under high (mimic treated) miR-200b conditions. Unsupervised clustering using CellRanger identified five cell clusters, three of which were responsive to changes in miR-200b abundance. Stable Isotope Labelling by Amino acids in Cell culture (SILAC) based proteomic analysis was employed to look for novel potential targets of endothelial miR-200b. A total of 3818 proteins were detected which were then filtered using statistical cut-offs (p value < 0.05; % change > 10%) identifying 319 proteins targeted by miR-200b. To determine the potential role of these candidates in regulating vascular function, miR-200b−429fl/fl-Tie2 Cre mice were generated where endothelial miR-200b levels could be specifically depleted in vivo. The therapeutic significance of endothelial miR-200b inhibition was studied using ischemic hindlimb in these mice. The significance of miR-200b depletion was also studied in streptozotocin-induced diabetic miR-200b−429fl/fl-Tie2 Cre mice. Perfusion imaging was performed using Laser Speckle imaging (Perimed Inc.) at different time-points (d3, d7, d10, d14). Inhibition of endothelial miR-200b rescued hindlimb ischemia with improved perfusion (n=11). Such effect of miR-200b inhibition on rescuing HL-ischemia was markedly increased in diabetic animals (n=8). Such rescue was associated with increased abundance of CD31+/VWF+ vasculogenic cells (n=6). This work identified miR-200b regulated endothelial cell clusters, the functional significance of which have been experimentally validated. These findings provide insight into novel mechanisms explaining how transient inhibition of miR-200b in the endothelial compartment improves angiogenic outcomes in the diabetic ischemic limb.

K3.02 | Tissue Nanotransfection Based Endothelial-Targeted Epigenetic Gene Editing In Vivo To Rescue Diabetic Ischemic Wounds

Sumit S. Verma, Surya Gnyawali, Chandan K. Sen, Kanhaiya Singh
Department of Surgery, McGowan Institute for Regenerative Medicine, University of Pittsburgh, Pittsburgh, PA

Our previous work (PMID: 35192691) has identified that genetically silenced phospholipase Cy2 (PLCy2) hinders VEGF therapy of the diabetic ischemic limb. Hyperglycemia caused hypermethylation of endothelial specific gene promoter. Given that epigenetic changes are reversible, this work tests the significance of gene-targeted therapeutic DNA demethylation in improving blood flow to diabetic ischemic wounds. Bipedicle ischemic wounds were placed in streptozotocin (STZ) induced acute diabetic C57BL6 mice. Diabetic VWF+ dermal endothelial cells were isolated using flow sorting. In wound tissue, PLCy2 promoter CpG methylation levels were analyzed using bisulfite sequencing. Next, to specifically demethylate PLCy2 promoter in endothelial cells, a demethylation cocktail was designed: (i) (scFv)-TET1 catalytic domain (TET1CD) system capable of inducing targeted DNA methylation, and (ii) endothelial promoter driven gene specific guide RNAs. This demethylation cocktail was delivered at the diabetic ischemic wound-edge employing non-viral topical tissue nano-transfection (TNT) technology. PLCy2 protein expression outcomes were assessed using flow cytometry. Functional outcome of such demethylation was assessed using Laser Speckle Perfusion imaging (Perimed Inc.) and ultrasonography (Vevo 2100) at different time-points post-surgery (days 3, 7,10). Overall DNA hypermethylation was prominent in murine ischemic flaps as demonstrated by the increased ratio of 5-methylcytosine (5-mc, methylation mark) to 5-hydroxymethylcytosine (5-hmC, demethylation mark) (n=5). Specifically, the PLCy2 promoter was hypermethylated (n=5). TNT mediated endothelial-targeted demethylation of the PLCy2 promoter increased the expression of this gene in endothelial cells (n=4). Such demethylation-based upregulation of PLCy2 improved wound tissue blood flow with increased abundance of VWF+/PLCG2+ vascular elements (n=4). Taken together, topical TNT-based endothelial demethylation of the PLCy2 gene promoter improved perfusion of cutaneous diabetic wounds resulting in improved closure.

K3.03 | AMPK and Rac1 Activity Regulation Promotes Wound Healing via Induction of Actin Cable Formation

Kento Takaya1, Yuka Imbe2, Qi Wang2, Shigeki Sakai2, Keisuke Okabe1, Noriko Aramaki-Hattori1, Kazuo Kishi1
1Plastic and Reconstructive Surgery, Keio University School of Medicine, Shinjuku-ku, Tokyo, Japan; 2Faculty of Pharmacy, Keio University, Minato-ku, Tokyo, Japan

Background: Unlike adults, early developing fetuses can completely regenerate tissue, and replicating this could lead to developing treatments to reduce scarring. Mice epidermal structures, including texture patterns, are regenerated until embryonic day (E) 13, leaving visible scars thereafter. Although the formation of actin cables at the wound margin and epidermal cell migration are known to be involved in this transition, the detailed mechanism remains unclear. We focused on AMP-activated protein kinase (AMPK) and Rac1, factors involved in regulating cell migration and actin dynamics, and investigated their effects on skin regeneration through regulation of AMPK and Rac1 activity using a unique mouse fetal wound healing model.

Methods: (1) Regulation of Rac1 activity: The mouse epidermal cell line PAM212 was treated with the Rac1 inhibitor NSC23766 and the effect on migration ability was evaluated by scratch assay. We generated genetically engineered mice (K14-CreERT2;Rac1fl/fta) that can suppress epidermis-specific Rac1 cell migration and observed the wound healing process and actin dynamics in fetuses and adults. (2) Regulation of AMPK activity: Keratinocytes were established from fetal mouse tissues and treated with AMPK activator salicylate, and the effect on migration ability was evaluated by scratch assay. Embryos of ICR mice E13, E14, and E15 were wounded and salicylate was administered into the amniotic fluid and collected at multiple time points. Wound morphology was analyzed by 3D reconstruction of the wound images, and the presence of actin cable formation and the behavior of related molecules were observed.
**K3.04 | A Novel Ex Vivo Human Fascio cutaneous Flap Perfusion Model to Investigate Skin Injuries**

Asim Ejaz  
*Plastic Surgery, University of Pittsburgh, Pittsburgh, PA*

Skin is the first line of defense against burns, chemicals, radiation, and trauma injuries. Recent research discovered a wide range of pathways and agents to treat skin injuries but still, there is a wide gap in the knowledge due to the complex nature of the injuries. Often, animal models are used for testing new agents, yet they lack anatomical feature resemblance to human tissue. Human tissue-based models are ideal; however, maintaining complex tissue ex vivo is challenging. Here we describe a novel, optimized, and well-characterized model of a full-thickness human skin perfusion system that utilizes surgical waste skin to cultivate flaps ex vivo. Abdominal panniculectomy samples were collected as surgical waste. Under sterile conditions, we isolated and cannulated perforators of the superficial and deep inferior epigastric systems. We perfused the cannulated tissue using a bioreactor system capable of real-time monitoring of pressure, flow rate, fluidic temperature, and tissue temperature. Albumin-supplemented culture media at 60mmHg pressure with 6ml/min inflow was perfused throughout the run time of approximately three weeks. Angiosome distribution was confirmed by fluorescein angiography and infrared imaging. Flow rate measurements, vascular reactivity, daily tissue biopsy samples for histology and electron microscopy, cell viability, lactate production, and gene expression levels were measured to assess the viability and proliferation run. Isolated adipose stem cells and dermal fibroblasts at 60mmHg pressure with 6ml/min inflow was perfused throughout the run time of approximately three weeks. Angiosome distribution was confirmed by fluorescein angiography and infrared imaging. Flow rate measurements, vascular reactivity, daily tissue biopsy samples for histology and electron microscopy, cell viability, lactate production, and gene expression levels were measured to assess the viability of the flap. Utilization of the skin perfusion model for chemical and burn injuries was assessed by induction of chemical (Nitrogen Mustard) and burn wounds. Samples were collected from the wounded and control tissue for histology, protein, and gene expression analysis. Angiography verified that the SIEA to SIEV flap system successfully fed ~90% surface area of a large flap for two-three weeks. Flow rate, temperature, and pressure remained steady throughout ex vivo cultivation. The vascular reactivity test showed a physiological response of the vasculature upon application of a vasoconstrictron (epinephrine) and vasodilator (papaverine). H&E staining and TUNEL immunofluorescence staining revealed healthy and viable cells during the perfusion run. Isolated adipose stem cells and dermal fibroblasts at different time points during perfusion showed viability and proliferation dynamics compared to fresh tissue isolates. We observed a decrease in circulatory glucose levels and increased lactate levels upon insulin challenge. Nitrogen mustard wounds showed a gradual increase in the dead TUNEL-positive cells. We observed the epithelial layer disruption and damage upon burn injuries. Our results suggest that this novel model system can keep the tissue viable for an extended period (app. 3 weeks) ex vivo. This viable system can help us understand the pathways and be used as a subclinical drug testing model.

**K3.05 | Subcutaneous Injection Of Zein, A Dietary Protein, Has Positive Wound Healing Effects That Are Prevented By Fingolimod (Fty720) Treatment**

Isabela Beatriz C. Nóbrega, Angélica Vitória S. Andrade, Geraldo Magela Azevedo, Claudia R. Carvalho  
*Morphology, Universidade Federal de Minas Gerais, Belo Horizonte, MG, Brazil*

Pathological scars resulting from impairment or modification in wound healing are serious problems for patients’ health and strongly impact the financial cost of their treatment. Pathological scars, such as hypertrophic scars and keloids, are common even in cases of scheduled surgery. Exacerbated and prolonged inflammation is an important factor in the occurrence of wound healing problems. The search for more effective forms of intervention that prevent pathological scars requires a better understanding of the cellular interactions that occur during repair. Previous work has shown that parenteral injection of dietary proteins, concomitant with skin lesions in mice, reduces the inflammatory infiltrate in the wound bed and improves healing. Zein is a corn protein present in mouse food and one injection of zein in Al(OH)₃ concomitantly with skin wounds reduces the inflammatory infiltrate, increases the number of T lymphocytes in the wound bed and improves healing (https://doi.org/10.1590/1414-431X2021e11735). To evaluate whether the positive effects of zein injection on the repair of skin lesions depend on the circulation of lymphocytes, we used FTY720 (Fingolimod), a drug that sequesters lymphocytes in lymph nodes. Male C57BL/6 mice, 8 weeks old, received intraperitoneal injections of 1mg/kg of FTY720 two days before injury, on the day of injury and two days after skin injury (CEUA/UFMG protocol 253/2021). Two lesions on the back skin were made with a 6mm dermatological punch and the animals in the experimental group also received one subcutaneous injection of 10 microgram of zein in 1.6 mg of Al(OH)₃, at the base of the tail. Skin analyses performed at 7 and 40 days after the injuries showed that, in the absence of FTY720, the injection of zein concomitantly with the injuries reduced the inflammatory infiltrate, increased the number of T lymphocytes in the wound bed and
improved the organization of the extracellular matrix in the neodermis. At 7 days after the injuries, it was noted that the injection of FTY720 delayed the detachment of the wound crust and increased the number of leukocytes (CD45+), neutrophils (Ly6G+) and macrophages (F4/80+) in the wound bed. Furthermore, FTY720 treatment altered the morphological characteristics of fibroblasts seen after H&E staining, suggesting greater activation of these cells. At 40 days after the injuries, the neodermis of animals that received injection of zein showed an organization of the extracellular matrix similar to that of intact skin but, on the contrary, those that received FTY720 and zein showed a larger scar, with thinner fibers organized parallel to the epidermis, with characteristics of a loose matrix and larger scar. In conclusion, FTY720 prevented the increase of T lymphocytes to the epidermis, with characteristics of a loose matrix and larger scar. In conclusion, FTY720 prevented the increase of T lymphocytes to the epidermis, with characteristics of a loose matrix and larger scar.

Predictable series of chemical and biological changes in each wound stage propel the healing process forwards. The catecholamines noradrenaline (NOR), epinephrine (EPI), and dopamine (DOP) are small molecule neurotransmitters present in the wound that can affect healing. Previously, we showed that physiological levels of EPI activate the alpha 2b adrenergic receptors (A2AR) on keratinocytes and accelerate their migration in vitro, while activation of beta 2 adrenergic receptors (B2AR) by supra-physiological levels decreased their migration (Yang et al., 2021). Thus, either changes in the wound concentration of epinephrine or in alpha/beta receptor expression may affect re-epithelialization. Here we examined catecholamines and related gene expression in the wound environment during normal wound healing in porcine skin wounds. Twelve full-thickness 20 mm diameter circular wounds were placed on 6 pigs and blood and wound tissue were sampled at intervals over 21 days. Wound outcomes were imaging, tissue RNASeq and histology, and reverse phase HPLC analysis of serum catecholamines. Wound image area and histologic re-epithelialization demonstrated that most wounds healed by days 16-21. Expression of the alpha 2a AR (ADRA2A) was immediately decreased following wounding and remained so through day 7 of healing; expression was increased on days 9 through day 21, peaking at day 16. Expression of the alpha 2b AR (ADRA2B) was bi-modal with peaks on days 2 and 13, and a gradual return to baseline on intervening days. Beta 2 AR (ADRB2) expression increased after wounding, peaking at day 2 and gradually returning to baseline by day 6 through day 21. Expression of dopa decarboxylase (DDC) only began to increase on day 7 of healing, peaking on day 11 and returning to baseline by day 21. DDC is essential to convert L-dopa to DOP, a known mediator of healing. Serum DOP remained at baseline through day 11 and then increased significantly through day 21, concurrent with elevated DDC expression in the wound. The concurrent peaking of DDC, ADRA2A, and ADRA2B suggest their role in enhancing keratinocyte migration in the latter half of the healing process. Serum EPI levels increased immediately after wounding and remained elevated through day 7, gradually returning to baseline by day 12, but effects could be mitigated by the concurrent increase of degrading enzyme monoamine oxidase B (MAOB) in the wound tissue. Likewise, the elevated expression of solute carrier family 6 a2 (SLC6A2, responsible for NOR reuptake) in the first half of the healing process could abrogate the anti-migratory effects of elevated ADRB2 expression in early healing. Taken together, these results show how the complex dynamics of the adrenergic network in the skin change during normal wound healing to support wound closure.

K3.06 | Changes In The Catecholamine Adrenergic Network
Support Wound Healing In Healthy Pigs

Anthony Gallegos3, Ksenia Zlobina1, Hsin-ya Yang3, Moyasar A. Alhamo3, Athena Souliska3, Mo Siadat3, Marcella Gomez1, Marco Rolandi3, Rivkah Isseroff3
1Applied Mathematics, UC Santa Cruz, Santa Cruz, CA; 2Electrical and Computer Engineering, UC Santa Cruz, Santa Cruz, CA; 3Dermatology, UC Davis, Sacramento, CA

K4: Bioengineering/Biomaterials

K4.01 | Type III Collagen Biomaterials Improve Cutaneous Wound Healing In Diabetic Mice

Daniel C. Stewart1, Yasumasa limori1, Becky K. Brisson1, William Yen1, David Chenoweth5, Claudia Loebel3, Jason Burdick5, Susan W. Volk1
1School of Veterinary Medicine, University of Pennsylvania, Philadelphia, PA; 2College of Arts and Sciences, University of Pennsylvania, Philadelphia, PA; 3College of Engineering, University of Michigan, Ann Arbor, MI; 4College of Engineering and Applied Science, University of Colorado Boulder, Boulder, CO

Background: Dysregulated collagen production and proteolytic degradation of collagen in diabetic wounds is well known to contribute to impaired healing in diabetic patients. Altered dynamic reciprocity between cells in the healing microenvironment in turn leads to altered epithelial, stromal, and immune cell responses after injury. Based on our previous work showing a vulnerable and pro-regenerative role for type III collagen (Col3) in acute wound repair, we hypothesized that delivery of Col3 to diabetic wounds could improve efficiency and quality of repair in a diabetic mouse model. Furthermore, we have developed a proteolytic-resistant Col3 (aza-Col3) which we hypothesize will further improve efficacy of Col3 biomaterials in diabetic wound healing.

Methods: Two 8 mm full-thickness excisional wounds were made paramedian on the dorsum of LeprDB/db (db/db) mice at 14-16 weeks of age. Shear-thinning, self-healing hydrogels were utilized to deliver rhCol3 (200 μg) or vehicle to the wound bed at the time of wounding. Wounds were collected and gross wound closure was assessed at 7-, 10-, and 14-days post-wounding (DPW). Histomorphometry was determined for total re-epithelialized distance (RE), epidermal gap...
(EG), and granulation tissue (GT) area on H&E sections. Immune responses were characterized by immunohistochemical staining for MPO and F4/80 (neutrophils and macrophages, respectively). rhCol3 degradation with or without aza-glycine peptides (aza-Col3) was assessed with circular dichroism (CD) spectroscopy in the presence of collagenases.

Results: We observed that Col3 biomaterial-containing wounds had improved wound healing as evidenced by increased RE at 7, 10, and 14 DPW compared to control biomaterial wounds (p<0.01 for each timepoint); decreased EG at 7 and 14 DPW (p<0.05); and increased GT formation at 10 DPW (p<0.05). At 10 DPW, Col3 biomaterial wounds had decreased neutrophil (MPO+ cells) and macrophage (F4/80+ cells) infiltration compared to controls (p<0.05 each). CD spectra revealed increased resistance to degradation in aza-Col3 samples dose-dependently with increasing aza-glycine concentration compared to rhCol3 alone.

Conclusions: Our results demonstrate that Col3 improves wound healing outcomes in a diabetic mouse model through its effects on re-epithelization, GT formation, and immune response. While the incorporation of Col3 alone improved wound healing outcomes, preliminary data also indicate that incorporation of aza-peptides with Col3 prevents degradation in the presence of collagenases and suggest that aza-Col3 biomaterials may further improve healing outcomes in diabetic wounds.

K4.02 | Intravesicular Cytokine Profiling Of Stage Iv Pressure Ulcers Treated With Npwt vs. Npwt and Porcine Extracellular Matrix Dressing

Lauren Fang1, Richard Simman2
1University of Toledo College of Medicine, Monroe, OH; 2ProMedica-Jobst Vascular Institute, Toledo, OH

Background: Extracellular vesicles (EVs) are involved in all phases of wound healing. They carry cytokines, which possess a wide range of pro-inflammatory and pro-healing functions. While inflammation is critical in wound healing, it must be resolved so that wounds can progress to proliferation and remodeling. Otherwise, wounds become chronic. The purpose of this study is to analyze intravesicular cytokines in wound fluid to understand how healing and non-healing wounds behave at the molecular level when treated with negative pressure wound therapy (NPWT) versus a combination of NPWT and Porcine Extracellular Matrix dressing (Oasis Ultra).

Methods: Wound fluid samples were obtained from 16 patients with stage IV trunk pressure ulcers. The patients were divided into two groups (n=8 in each): a control group on NPWT alone and a study group on NPWT plus Oasis Ultra. A canister of patient wound fluid was collected from the NPWT device (wound VAC) every four weeks over the course of the 12-week study. Microvesicles were isolated and analyzed for concentration and content. The following were assayed: growth factors, proinflammatory interleukins and molecules, anti-inflammatory interleukins, and enzymes.

Results: In previous experiments, we found that the overall wound healing rate was significantly higher in the Porcine Extracellular Matrix study group compared to the control group (p<0.05), as determined by wound size at 12 weeks. Study group wounds also healed faster. In our molecular data analysis, wounds in the study group expressed higher intravesicular pro-healing growth factor concentrations earlier in the study compared to wounds treated with NPWT (control) alone. For example, the intravesicular fibroblast growth factor (FGF) concentration at 4 weeks in the study group was statistically significantly higher than the control group (112.93 pg/mL vs. 28.51 pg/mL, p<0.01). As wounds progressed toward healing, the EV concentration of proinflammatory molecules such as IL-5, IL-6, IL-8, IL-12, IFN-γ, and TNF-α decreased in the study group over time and were lower than control group levels by 12 weeks. Anti-inflammatory molecules IL-1ra and IL-10 were produced at higher concentrations in the faster healing study group as compared to the NPWT only group.

Conclusion: Wound healing is a regulated process involving both proinflammatory and inflammation-resolving mediators. Given the correlations between our molecular data and prior clinical wound healing data, our results suggest critical interactions between intravesicular cytokines and cells in the actual wound site. To our knowledge, this is the first study to successfully isolate microvesicles containing wound healing specific cytokines at multiple time intervals for a prolonged period. Intravesicular molecular profiling can help characterize wound progression, serving as a wound healing biomarker.

K4.03 | Laser Micropatterned Dermal Templates Improved Cultured Epithelial Autograft Handleability And Development In Vivo

Britani Blackstone1, Molly E. Baumann1, Summer Gallentine1, Dorothy Supp1, Kevin Bailey2, Heather Powell1
1Materials Science and Engineering, The Ohio State University, Columbus, OH; 2Surgery, Wake Forest University, Winston Salem, NC; 3Dermatology, University of Cincinnati, Cincinnati, OH

The success of cultured epithelial autografts (CEAs) as a treatment for large surface area burns is limited by graft fragility and resulting issues with handleability and blistering. Rete ridges play a significant role in epidermal adhesion; however, they are commonly missing from cultured skin grafts and CEA-treated wounds for up to one year. The purpose of this study was to explore whether a fibroblast-seeded dermal template (DT), fabricated with a dermal papillae-like architecture, could facilitate faster rete ridge development and improve the outcomes of CEA application in vivo. A porcine burn-excite-autograft model was used to establish a viable, partial thickness wound bed for grafting of CEAs alone or in conjunction with a flat (CEA-Flat) or micropatterned (CEA-Ridged) DT. Porcine keratinocytes and fibroblasts were isolated from split-thickness skin grafts from four subjects. Keratinocytes for each pig were cultured for 20 days to form CEAs. After expansion, fibroblasts from all pigs were pooled and seeded onto disinfected, hydrated electrospun collagen scaffolds to form...
DTs. A fractional carbon dioxide laser was used to micropattern the surface of half of the DTs prior to combination with the CEAIs. For 9 weeks, grafts were assessed for contraction, epidermal barrier function, pigmentation, biomechanics, basement membrane formation and vascularization. Combinatorial use with a DT improved handleability and graft integration at the margins, and speeded restoration of epidermal barrier function by 2 weeks. At 2 weeks post-grafting, increased epidermal proliferation and localization of collagens IV and VII near the dermal-epidermal junction were observed in CEA grafting, increased epidermal proliferation and localization of collagens IV and VII near the dermal-epidermal junction were observed in CEA +Ridged grafts, over CEA alone and CEA+Flat groups. Additionally, rete ridges were observed in all CEA-Ridged grafts from week 2, while these features were less frequent and shallow in CEA alone and CEA+Flat grafts over the course of the study. No differences between the groups were found in graft contraction or post-grafting biomechanics, and a stiffer substrate may better mitigate graft contraction for future use with epidermal sheets or suspensions. Overall, inclusion of a laser micropatterned dermal template improved graft development and decreased variability overall.

K4.04 | Sustained Oxygenation And ROS-Scavenging By Lignin Composites Promote Diabetic Wound Healing

Tanuj J. Prajapati1, Lane Yutzy2, Oluyinka Olutoye1, Benjamin Padon1, Walker D. Short1, Fayiz Faruk1, Sonya S. Keswani1, Nabila N. Anika1, Olivia Jung2, Phillip Kogan1, Ling Yu1, Hui Li1, Jangwook Jung2, Swathi Balaji1

1Pediatric Surgery, Texas Children’s Hospital and Baylor College of Medicine, Houston, TX; 2Department Of Biological Engineering, Louisiana State University, Baton Rouge, LA

Excessive reactive oxygen species (ROS) potentiate inflammation and impair neovascularization resulting in impaired diabetic wound healing. We engineered novel lignin (a natural antioxidant from lignocellulose)-based composites with ROS-scavenging and oxygen-releasing properties and hypothesized that they enhance neovascularization and attenuate inflammation and fibrosis to promote diabetic wound healing. Injectable lignin composites were prepared in methacrylated gelatin with test groups including antioxidant nanoparticles (Thiolated Lignosulphonate-TLS), antioxidant with O2 generation via incorporation of CaO2 in the nanoparticles (CPO) and its control (CPOc), and untreated. Full-thickness 6mm stented wounds were made in db/db (8-10 wk, F/M) mice and treated immediately on d0. Wounds were examined for epithelial gap (K14), granulation tissue (H&E), endothelial cells and lumens (CD31), VEGF and HIF1α, leukocytes (Ly6g,CD45) and macrophage (F4/80,CD206, arginase1) at d7 and d14. Weighted Gene Co-expression Network Analysis (WGCNA) was used to perform correlation network analysis on gene sets from a fibrosis PCR array data from dermal fibroblasts cultured on lignin composites. p by ANOVA.

In db/db skin wounds, CPO composites promoted wound closure and granulation tissue deposition (p<.05) and CD31+ capillary lumen density at d7 (p<.01). VEGF expression in wound homogenates was also significantly higher at d7 (p<.05), suggesting CPO composites promote angiogenesis in diabetic wounds. Interestingly, decreased HIF1α expression was noted in the leading wound epithelium, with reduced expression in wound beds in CPO wounds (p<.05). CPO wounds also had reduced IL-6 (p<.05) and macrophage infiltration while maintaining the highest proportion of CD206 and arginase1 dual stained macrophages that are pro-healing. We then determined the effect of the lignin composite treatment on wound remodeling at d14. Improved healing in CPO wounds was supported by the presence of a robust granulating wound bed (H&E), along with an increase in the CD31+ lumens. ClueGO functional enrichment analysis revealed thyroid stimulating hormone as a key hub gene from fibroblasts cultured in vitro on lignin composites. Since thyroid hormone receptors regulates TGF-β signaling, specifically, the binding of thyroid hormone triiodothyronine (T3) through nuclear receptors regulating the TGF-β/SMAD pathway, our findings indicate a role for lignin composites in governing TGF-β signaling to attenuate fibroblast fibrotic responses. Our data showed that the dual function of antioxidation and oxygen production capacity of the lignin composites improved wound healing associated with enhanced neovascularization and reduce inflammation, representing new frontiers in improving diabetic wound healing by engineered biomaterials.

K4.05 | In-Situ Bioprinting With Pro-Reparative Bioinks Improves Impaired Diabetic Wound Healing

Eleftheria Angeliki Valsami1, Seol-Ha Jeong2, zhuqing li1, Enya Wang1, Jihyun Kim2, Lance Fiondella3, Su Ryon Shin2, Aristidis Veyes1, Georgios Theocharidis1

1Beth Israel Deaconess Medical Center, Boston, MA; 2Brigham and Women’s Hospital, Boston, MA; 3Electrical and Computer Engineering, University of Massachusetts-Dartmouth, Boston, MA

Existing in-situ printing techniques exhibit critical challenges, such as time-consuming calibration and restricted adaptability, limiting their application in clinical settings. We present INSIGHT (INtelligent Guiding robot Technology), an innovative Artificial Intelligence driven system that identifies arbitrary areas at various angles without predesigned code and calibration procedures. Diabetic foot ulcers is a substantial problem for patients and clinicians. In this study we hypothized that we could harness advanced bioinks containing oxygen releasing particles and human mesenchymal stem cells (MSCs) to improve wound repair by regulating wound microenvironment and inflammatory response. MSCs are key cells in all phases of wound healing. We performed in vivo and in vitro studies to evaluate INSIGHT’s application of diverse bioinks on diabetic wound healing. 2 wounds were created on the dorsal skin of db/db mice and real_time scanning allowed for automatic generation of printing paths specific to each wound. Three different groups of microgels were studied: Gel, gel with oxygenating microparticles...
Magnesium (Mg) is a co-factor for critical enzymes important in wound healing. Biodegradable Mg metal alloys can promote wound healing by releasing Mg ions. We optimized alloy chemistry and thermomechanical processing conditions to manufacture fine Mg wire with sufficient mechanical properties to withstand wound implantation and in-service loading with excellent tissue tolerance. We hypothesize that Mg metal devices will provide physical guidance during early phases of wound closure and promote neovascularization, neuronal growth, and wound regeneration.

Mg alloy wires (WE43B, 127 μm, 90% cold work, and 250°C heat treated) were cut to 6mm length, and 5 wires were placed in 6mm stented full-thickness flank skin wounds in C57BL/6J mice (n=6, F, 8 weeks). Contralateral flank wound was treated with PBS as an internal control. Wound sections were stained with H&E to measure epithelial gap and granulation tissue at d7: neovascularization (CD31), leucocyte and macrophage infiltration (CD45; F4/80), and neurons (tuJ1) were assessed at d7. Scar area, collagen density, and lumen density, epithelial thickness and dermal appendages were analyzed at d28. MicroCT was performed at d7 and d28 to see Mg wires. p values by ANOVA.

All mice tolerated Mg wire placement. Gross observation showed no difference in exudate compared to PBS. Mg wires were visible at d7 and microCT revealed very small fragments even at d28, suggesting Mg wire degradation was appropriate to support all phases of wound healing. At d7 there was no difference in epithelial gap closure, but Mg significantly improved granulation tissue (0.5±0.19 vs 0.29 ±0.09 mm2, p<0.001). Mg also reduced inflammatory cell infiltration of both leukocytes (21.4±4.3 vs 34.1±5.1 cells/HPF, p<0.01) and macrophages (27.1±4.1 vs 50.1±7.5 cells/HPF, p<0.01), and improved wound neovascularization (21.2±5.7 vs 12.7±4.3 lumens/HPF, p<0.01) and tuJ1 fluorescence expression compared to PBS. At d28, a very small scar remained in Mg wounds. Scar area was reduced in Mg wounds, with improvement in ECM organization and subepithelial nuclear counts in papillary dermis, significantly increased lumen density (16±3.6 vs 11.5±1.7 lumens/HPF, p<0.05) and dermal appendages.

Our data demonstrate that Mg metal wires reduce inflammation and promote granulation tissue formation, neovascularization, and neuron growth early in wound healing to support regenerative dermal wound healing. This provides a strong rationale to harness Mg metal use in wound healing applications, especially to treat infected or chronic wounds without creating adverse responses such as antibody resistance or rejection of the treatments.
Diabetic foot ulcers are a significant health care burden. Current diagnostic approaches are primarily limited to symptomatic assessment and wound size monitoring, and new diagnostic tools are lacking. Therefore, there is a need for non-destructive imaging methods capable of characterizing and predicting healing outcomes. We have previously demonstrated that label-free multiphoton microscopy (MPM) can serve as a non-invasive diagnostic tool for skin wounds. Through MPM, we can visualize the autofluorescence of metabolic cofactors NADH and FAD, as well as collagen organization in 3D at a high-resolution. We have shown an optical redox ratio of FAD/(NADH + FAD) fluorescence intensities of the wound epithelium is sensitive to both diabetes- and age-related delays in healing. However, the interaction of these comorbidities on the epithelial redox ratio has not been elucidated. The goal of this study was to quantify the interaction of diabetes and age effects on wound closure and metabolism using in vivo label-free MPM.

Young (5 mo.) and aged (24 mo.) streptozotocin-induced diabetic (n = 17; 50/50 sex split) C57BL/6J mice received full-thickness, excisional wounds on their dorsal. Wounds were imaged longitudinally over 10 days using a multiphoton microscope that collected NADH (755nm excitation/460nm emission) and FAD (855nm excitation/525nm emission) autofluorescence. Image z-stacks were acquired at three wound edge locations using a 20x, 1.0 NA objective. Wound boundaries were manually traced to evaluate the rate of closure. Changes in the optical redox ratio within the epithelium were assessed using a multi-factor ANOVA and posthoc Tukey’s HSD tests. Wound size measurements indicated that closure was most delayed in aged diabetic mice, while closure was fastest in young non-diabetic controls. The epithelial redox ratio at the wound edge changed over 10 days similar to previous studies. The epithelial redox ratio of young non-diabetic control mice significantly decreased (p < 0.0001) by day 3 during the initial inflammatory phase, which then increased during re-epithelialization, allowing the redox ratio to return to baseline levels by day 10. Aged control mice, however, did not exhibit a significant change in wound metabolism until day 7 (p < 0.0001), suggesting delays during the inflammatory phase. The epithelial redox ratio of young and aged diabetic mice significantly decreased by day 3 (p < 0.0029) like young control mice, but did not increase at later time points. While not statistically different from other groups, aged diabetic mice had the lowest redox ratio at day 10. In conclusion, these findings suggest that in vivo label-free MPM is sensitive to diabetes-induced delays in healing, independent of the metabolic effects associated with advanced age.

Jelena Marjanovic1, Jamie L. Burgess3, Ivan Jozic1, Beatriz Abdo Abujamra1, Robert S. Kirchner1, Hadar Lev-Tov1, Harold Brem2, Momoh Ojirese2, Irena Pastar1, Marjana Tomic-Canic1

1Dr. Phillip Frost Department of Dermatology and Cutaneous Surgery, University of Miami Miller School of Medicine, Miami, FL; 2Rutgers New Jersey Medical School, Newark, NJ

Venous leg ulcers (VLUs) represent one of the most prevalent types of chronic wounds yet only 44.1% of VLUs heal with standard of care, underscoring the critical need to better understand the molecular and cellular pathology of VLUs. This study focused on multi-omic approaches to identify novel therapeutic targets and key cellular and molecular mechanisms that are associated with clinical outcomes of
VLU healing. Patients with chronic VLU were recruited from 2 clinical centers. To identify the unique non-healing gene signatures, we performed bulk and single cell RNA sequencing on healing (n=5) and non-healing VLUs (n=6) and utilized Ingenuity Pathway Analysis to perform comparative genomics to human acute wounds (AW) collected at day 3. Immune response of healing VLUs showed a high resemblance to acute wounds, while non-healing VLUs signature showed a strong suppression of the cellular inflammation and, much less studied, lymphangiogenesis. We confirmed these findings in prospectively collected tissue (n=4 per group) by immunostaining. We demonstrated a significant decrease in CD45, CD3, CD4, CD19, CD68 and podoplanin positive cell population in non-healers compared to healers. Further, the absence of a proper inflammatory response was a consequence of multiple impaired cellular processes: suppressed transmigration, improper chemotaxis, and immune cell recruitment, which was corroborated by significant suppression of CCL4, CCL13, CXCL9, CXCL12, CX3CL1, ICAM1, ITGB2, VCAM1, TLR4, and XCR1 in non-healing VLUs. To identify the molecular mechanisms contributing to a non-healing VLU signature, we performed proteome profiler arrays and found suppression of AKT, ERK, p38 MAPK, SRC, STAT and PDGFR signaling pathways in non-healers when compared to healers. Upstream regulator analyses pointed to PTEN as a master negative regulator of all these pathways. PTEN protein analyses showed significant induction in non-healers when compared to healers. Indeed, PTEN inhibitor accelerated wound closure in murine wounds in vivo. In addition to increasing immune cell response, PTEN inhibitor also induced IFNγ and CXCL13. Thus, we identified PTEN as a master regulator of non-healing phenotype of VLUs that orchestrates impaired immune response and lymphangiogenesis. Identifying PTEN signaling and cellular makeup that is critical in promoting VLU healing provides key targets for novel therapeutic approaches that will successfully shift a non-healing to a healing VLU and promote closure.

Methods: We created full-thickness excisional wounds on the dorsum of C57/BL6 (WT) mice, WT mice subjected to a high-fat (HF) diet to induce pathophysiologic diabetes, and leptin-receptor deficient (DB) mice with genetically induced diabetes. Mice were sacrificed at post-operative days (POD) 0, 2, 7 and 30. Tissues were processed into single-cell suspensions for single-cell RNA sequencing. Bioinformatic analyses were used to identify and further analyze fibroblasts (Col1α1+, Col1α2+, Col5α1+, and Vim+).

Results: The focused analysis of fibroblasts revealed nine distinct sub-populations defined by a unique gene expression profile, indicative of its functional characteristics, including fibrotic (F), keloid-initiating (KI), altered epithelial-like (AE), and angiogenic (A) clusters. Notably, on POD2, WT fibroblasts demonstrated considerable enrichment in A (12%) and F (14%) clusters, while HF exhibited a deficiency (<1%) in both. Contrarily, DB only displayed a slight growth in A (3%), and F (4%). Across the different conditions at POD2, KI cluster displayed zero growth. At POD7, WT increased expression in A (15%), and declined in F (10%). These cells also demonstrated a sharp growth in KI (20%). DB rose to 8% in A and skyrocketed in F and KI (26% and 35% respectively). On the other hand, DF displayed a marginal increase of 3% in A and 1% in F with a substantial increase in KI (10.5%). Interestingly, DF displayed a significant increase in the AEL subpopulation with 40% vs <0.1% in DB and WT. By POD30, A, KI and AE subpopulations dropped back to <0.7%. F remained marginally expressed in DF and HB at both 2% and WT regressed to 0%.

Conclusion: This study discovered distinct temporal patterns of A, F, AEL, and KI subpopulations of fibroblasts within WT, HF, and DB mice. Interestingly, A and F subpopulations were significantly weakened in the diabetic groups at POD2. At POD7, unique HF AEL clusters may critically affect impaired diabetic healing. Proportionally, the number of fibroblasts decreased by up to 100-fold by POD 30 compared to POD 7, demonstrating a resolution of the wound healing process. Creating therapies to increase A and fibrotic F subpopulations could improve diabetic wound healing.

L1.04 | Transcriptional Analysis of Fibroblasts in Diabetic Wound Healing

Abdelrahman Alsharif, Katharina S. Fischer, Filiberto Quintero, Mansi Singh, Amelia B. Knochel, Ben Litmanovich, Sultana M. Mojadidi, Javier Gonzalez, Dharshan Sivaraj, Hudson C. Kussie, William Hahn, Andrew Hostler, Maia Granoski, Geoffrey C. Gurtner, Kellen Chen

Department of Surgery, University of Arizona, Tucson, AZ

Background: Diabetic wound healing poses a significant public health concern, impacting approximately 30 million individuals in the United States alone, with projections indicating a doubling by 2050. The cellular mechanisms that prevent proper wound healing are still not fully understood. Fibroblasts are crucial contributors to all stages of wound healing. Given their significance, this study is dedicated to a comprehensive exploration of how heterogeneous populations of fibroblasts contribute to diabetic wound healing using transcriptomic analysis and various different mouse models over time.
ulcers (VLUs), showed the efficacy of BBD in WBP. The purpose of this analysis was to assess the correlation between WBP and wound closure (WCL).

Methods: In the ChronEx study patients with chronic VLUs were randomized (3:2:2 ratio) to daily treatment with either BBD, placebo gel vehicle, or non-surgical standard of care (NSSOC), for up to 2 weeks or until reaching complete debridement. Patients were then followed-up weekly with standardized NSSOC for 12 weeks. WBP was defined as complete debridement of non-viable tissue and wound bed completely covered with granulation tissue, both assessed clinically. Complete WCL was defined as complete re-epithelialization of the wound surface without drainage or dressing, confirmed at two visits. The incidence of WCL during the study was compared between those who achieved or did not achieve WBP, in all randomized patients. Incidence of WCL during the follow-up was compared between patients who achieved or not WBP by 16 days (end of daily treatment). The correlation between time to WBP and time to WCL was assessed using a time-dependent proportional hazards Cox regression model.

Results: A total of 119 patients were randomized to the study, with an average wound size of 15.5 cm² (SD19.4), average non-viable tissue of 73.2% (SD15.2) and wound present for an average of 31.2 weeks (SD23.9).

Overall, 80 patients (67%), reached WBP anytime throughout the study and 39 (33%) did not. Of those that reached WBP, 34 patients (42.5%) achieved WCL within the study period, while only 4 (10.3%) of the patients that did not achieve WBP achieved WCL, with relative risk (RR) of 4.1 (95%CI=1.58-10.85, p=0.0004).

Patients reaching WBP by 16 days were 2.2 more likely to achieve WCL within 12 weeks follow-up compared to patients who did not reach WBP by that time, (50.0% vs. 22.7%, 95%CI for RR 1.31-3.71, p=0.0028).

Time to WBP correlated with time to WCL (hazard ratio-11.96, 95% CI= 4.24-33.79, p<0.0001).

Conclusions: The ChronEx study demonstrates that wound bed preparation of chronic VLUs significantly increased the likelihood of complete WCL. These findings support the critical importance of adequate WBP in the process of wound healing. A future phase 3 pivotal study with BBD will utilize these new data to assess the efficacy of this novel treatment in early facilitation of active WCL in VLU.

L2: Novel Therapies

L2.01 | Deep Vein Thrombosis Affects Healing Outcomes In Patients With Pyoderma Gangrenosum: A Single-Center Prospective Case-Control Study

Hannah Zhao, Sidharth Sengupta, Jonathan Sisley, Olivia M. Haddadin, Alex Ortega-Loayza

OHSU, Lake Oswego, OR

While inflammation and thrombosis are closely integrated processes, there has been little exploration between PG and thromboembolism. In this single-center case-control study, we assess the association between a history of DVT and healing outcomes in PG patients.
This prospective case-control study was approved by the OHSU IRB and was conducted between December 2019 and July 2023. Inclusion criteria was defined as patients with classic, ulcerative PG of the lower extremity confirmed by a PARACELSUS score of ≥10 from the Pyoderma Gangrenosum Study Registry (PYGAS) at OHSU. Data regarding patient demographics, comorbidities, and healing outcomes were extracted for further analysis. Patients were then grouped based on the presence or absence of ultrasound-proven lower extremity DVT prior to PG diagnosis. Cox regression and Kaplan-Meier survival analysis were applied to detect the effect of DVT on healing status within a 1-year period from initial presentation. Chi-squared tests were used to compare the descriptive statistics of the DVT and non-DVT groups. Statistical significance was established as P ≤0.05.

In total, 99 patients met inclusion criteria, with 13 (13.1%) having a history of DVT and 86 (86.9%) without. 121 total ulcers were included in the analysis—patients presenting with sequential or recurrent ulcers were recorded as separate encounters. The mean age of presentation for patients with and without DVT was 57 (SD +/-11) and 53 (SD +/-18), respectively (P=0.60). There were significantly more males presenting with DVT than females (P =0.04). There were no significant differences in race, ethnicity, or smoking history between the two groups. There were also no significant differences in confounding comorbidities such as body mass index, history of solid or hematologic malignancy, or history of autoimmune or inflammatory disorder between the DVT and non-DVT groups. Evaluation of median healing time between DVT and non-DVT groups using Kaplan-Meier survival analysis confirmed a significant increase in median healing time among patients with a history of DVT. The median healing time for the DVT and non-DVT group was 224 and 145 days, respectively (P=0.04). Univariate and multivariate Cox proportional hazard modelling was also used to assess the influence of various predictor variables on wound healing. When adjusting for obesity and smoking, the two factors most prevalent in our patient population and factors known to influence wound healing, a history of DVT remained the most significant influence on the healing time (P=0.05). Single predictor modelling also demonstrated that variables such as diabetes mellitus, solid malignancy, and multi-ulcer presentation did not significantly alter healing time. In conclusion, these findings suggest a novel association between a history of DVT and poorer healing in patients with PG, indicating the importance of identifying a history of thromboembolism when evaluating PG patients.

**L2.02 | Selective Agonism Of Histamine Receptors Augments Tissue Repair**

Jordan R. Yaron, Shubham Pallod, Nicole Grigaitis, Samantha Rhodes, Dirghau M. Patel, Deepanjan Ghosh, Kaushal Rege

*Biodesign Center for Biomaterials Innovation and Translation, Arizona State University, Tempe, AZ*

**Purpose:** Histamine is produced by mast cells and non-canonically by other cells during tissue injury. Four G-protein coupled receptors for histamine (HRH1-4) regulate diverse vascular, fibrotic, and immune signaling. Histamine signaling is critical to healthy wound healing and is dysregulated in diabetic wounds. To understand the role of specific histamine receptors during tissue repair, we performed a systematic investigation of HRH agonism during wound closure in vitro and biomaterial-assisted incisional wound repair in vivo.

**Methodology:** Incisions were made in Balb/c mice and sealed with silk fibroin-based laser-activated sealants (LASE) with codelivery of saline, histamine, or selective agonists for HRH1 (2-pyridylethylamine), HRH2 (dimaprit), HRH3 (immethridine), or HRH4 (4-methylhistamine), hereafter referred to as HRHn-ag (where n is 1-4). Trans-epidermal water loss (TEWL) was measured daily for 3 days. Ultimate tensile strength (UTS) was measured after histamine treatment. IHC was performed for HRH1-4 post-wounding, and for M1/M2 macrophage and neutrophil infiltration, angiogenesis, proliferation, and EMT. Expression of HRH1-4 in HaCaT keratinocytes was evaluated by immunofluorescence and western blot. Migration in response to histamine or agonists was performed by scratch assay.

**Results:** Histamine treatment of LASE-sealed incisional wounds results in a significant early increase in UTS without alteration of TEWL vs. saline-treated wounds. In contrast, selective agonism of HRHs results in diverse TEWL responses: HRH1-ag (N=3) increased TEWL (p<0.05), HRH2-ag (N=3) and HRH4-ag (N=3) decreased TEWL (p<0.05), and HRH3-ag (N=4) causing an initial increase in TEWL (p<0.001) with a return to physiologic levels after day 1. We found that HaCaT cells express all HRHs. Interestingly, while histamine (p<0.01) and HRH1-ag (p<0.0001) accelerate scratch closure, HRH2-ag almost completely prevents migration (p<0.0001). IHC for tissue responses during repair indicated a pro-resolution macrophage environment (high Arg1, low CD86) with HRH1-ag and HRH4-ag (p<0.01), and a milder response by HRH2-ag (p<0.05) and no response by HRH3-ag. HRH2-ag and HRH4-ag robustly suppressed neutrophil presence (p<0.0001), with a milder response by HRH1-ag (p<0.01) and no response by HRH3-ag. Angiogenesis (CD31+ vessels) was stimulated by HRH1-ag (p<0.01) and HRH4-ag (p<0.0001), with no response by HRH2-ag and HRH3-ag. Epidermal EMT (loss of E-cadherin integrity) was promoted by HRH1-ag (p<0.05) and suppressed by HRH2-ag (p<0.01), with no response by HRH3-ag and HRH4-ag. Keratinocyte proliferation was unaffected by all receptors.

**Conclusion:** HRH1 and HRH4 activation drive a pro-resolution immune response with enhanced angiogenesis, while HRH1 also stimulates increased epidermal EMT. HRH2 activation slows epidermal healing, and HRH3 activation has minimal effect on healing.

**L2.03 | Preclinical And Clinical Development Of Sli-F06, A Novel Dermal Fibroblast Modulating Drug, In Cutaneous Wound Healing**

ZHONG ZHENG1, Pin Ha2, Elisabeth Leeflang1, Zhaohan Zeng1, Joshua Yang2, Alyssa Miao2, Robert Galiano3, Paul Glat4, Donald Buck5, John Felder6, Kang Ting7, Chia Soo8

1Scarless Laboratories Inc., Cherry Hill, NJ; 2University of California, Los Angeles, Los Angeles, CA; 3Northwestern University, Chicago, IL; 4Dr. Paul Glat Clinic, Bala Cynwyd, PA; 5American Dental Association Forsyth Institute, Cambridge, MA; 6Washington University, St. Louis, MO
SLI-F06, a fibromodulin (FMOD)-based peptide, enhances wound healing. In comprehensive animal models, including mice, rats, and Yorkshire pigs (a gold standard for normal human wound healing), SLI-F06 exhibited FMOD’s pro-migration, pro-tensile strength, and anti-fibrotic properties. Here, red Duroc pigs, which more closely mimic human hypertrophic scarring, were wounded to further assess the translational potential of SLI-F06 to humans. Large ellipses were designed to create excessive-mechanical-loading (EML) to represent a high-tension wound potential of SLI-F06 to humans. Large ellipses were designed to create hypertrophic scarring, were wounded to further assess the translational potential of SLI-F06 to humans. Large ellipses were designed to create excessive-mechanical-loading (EML) to represent a high-tension wound relative. Primary closed wound edges were immediately injected with 10 mg/ml SLI-F06 or triamcinolone acetonide (TAC), a commonly used corticosteroid for repressing scar. At 8 weeks, TAC did not considerably improve gross scar appearance or reduce scar size, while it significantly reduced scar tensile strength. Meanwhile, SLI-F06 markedly improved visual scar appearance (P < 0.0001; N = 12) and significantly reduced scar size, especially in the EML wounds (resulting in 56% Scar Index reduction; P = 0.0024; N = 5). More importantly, SLI-F06 resulted in a 29% and 160% tensile strength increase in the normal (P = 0.0066; N = 6) and EML wounds (P < 0.0001; N = 6), respectively. Nonclinical safety studies include a 5-day and a 28-day intravenous bolus repeat-dosing in rats, a 5-day subcutaneous repeat-dosing in pigs, and a 3-day intradermal repeat-dosing in a wounded pigs. All toxicology studies demonstrated no observable adverse effects at maximum feasible doses. Ames and Epskin testing have shown no genotoxicity or local irritation, respectively. After FDA clearance, we performed a multicenter, double-blind, First-in-Man study to compare the safety of the cGMP-compliant SLI-F06 drug product to control formulation buffer (vehicle). Twenty-four (24) subjects were enrolled and 21 completed the study. Each subject served as his or her own control. The study was divided into 2 parts: Part A was a safety and proof-of-concept study of small scars pre-abdominoplasty, and Part B was a phase Ila study of post-abdominoplasty scars. There were no drug-related adverse events or clinically meaningful abnormal laboratory values, and all subjects were negative for antibiotic antibodies in immunogenicity testing. Moreover, the POSAS assessment indicated that the non-optimized, low-dose, one-time injection of SLI-F06 led to a 24.6% Surface Area improvement at the high-tension abdominoplasty wound segments (P = 0.0405; N = 42). Therefore, SLI-F06 shows promising preliminary efficacy in minimizing cutaneous scarring and excellent tolerability in human subjects, while further refinement of dosage and treatment regimens are essential for optimal results.

**L2.04 | Prophylactic, One Time Dose Of Rac Inhibitor Mitigates Foreign Body Response Through Immunomodulation At Both Early And Late Time Points**

Hudson C. Kussie, Jonathan P. Yasmineh, Dharshan Sivaraj, Katharina S. Fischer, Eamonn McKenna, Brodl Stevens, Gabriel Starling, Maia Granoski, Andrew Hostler, Maisam Jafri, Geoffrey C. Gurtner, Kellen Chen

*University of Arizona, Tucson, AZ*

**Purpose:** Our study explores how a single prophylactic dose of Rac inhibitor can significantly diminish the foreign body response around biomedical implants, potentially improving the efficacy and durability of such devices in patient care.

**Methods:** We used our previously published mechanically stimulated implants (MSIs) (Padmanabhan et al, 2023, Nat Biomed Eng) to create severe, pathological human FBR in mice. These MSIs were made of polydimethylsiloxane (PDMS) and encapsulated coin motors connected to external 3V batteries to initiate mechanical stimulation through vibration to create significant FBR fibrosis. Mice either received saline only (vehicle) or a prophylactic 5mg/kg NSC Rac inhibitor dose. After 7 days of vibration (POD 11), samples from 5 no vibration (NV), 5 MSI with saline (MSI-S) and 5 MSI with Rac Inhibitor (MSI-RI) were collected. After a month (POD30), 5 NV, 10 MSI-S, and 10 MSI-RI samples were collected. For this explanted tissue, FBR tissue was analyzed using staining including picrosirius red, hematoxylin and eosin, trichrome, and immunohistochemistry (IHC) for aSMa, F4/80, and DAPI.

**Results:** At POD 7, MSI-S significantly increased FBR compared to NV (p = 0.0465), and MSI with Rac Inhibitor treatment significantly reduced FBR by 47% from 284.8 to 133.8 μm compared to MSI-S (p = 0.03) to be close to NV levels. Similarly, at POD30, MSI-S significantly increased FBR compared to NV (p = 0.005), and MSI with Rac Inhibitor treatment significantly reduced FBR by 51% from 370.2 to 183.2 μm compared to MSI-S (p = 0.0008), and not significantly different than NV levels. At early time points, MSI-S significantly reduced the length (p = 0.037) while increasing alignment (p = 0.001) and density (p = 0.004) compared to NV. MSI-RI restored these collagen metrics (p = 0.016, p = 0.104, p = 0.001) back to NV levels. At late time points, these effects were blunted. Using trichrome imaging, distinct architecture layers were evident in the MSI-S capsule, with a unique inner layer that had less wide, dense, and aligned collagen fibers (p = 0.0020, p = 0.0078, p = 0.0051, respectively) compared to mechanically inhibited FBR which possessed no layering. Finally, we observed that Rac inhibition significantly reduced the number of macrophages (F4/80, p = 0.02) and myofibroblasts (aSMa, p = 0.03) compared to MSI-S.

**Conclusions:** Our study demonstrates that disrupting mechanical signaling with a one time prophylactic dose of Rac inhibitor notably reduces FBR capsule size and fibrotic ECM architecture. We observed a previously unreported layering in severe, pathological FBR, which seemed to be linked with distinct layers of macrophages and fibroblasts within the capsule. Disrupting mechanical signaling with a one time, prophylactic therapeutic could have clinically meaningful effects for patients, and future biomedical implants could even be coated in a one time dose of this drug.

**L2.05 | Comparison Of Bromelain-Based Enzymatic Debridement To Collagenase Satyl® Ointment - Analyses From The Chronex Multicenter Rct**

Dove Çyaandil, Robert Snyder, Keren David, Yael Katz-Levy, Ety Klinger, Felix Sigal

1. University Health, San Antonio, TX; 2. Barry University, Miami, FL; 3. MediWound Ltd, Yavne, Israel; 4. Angel City Research, Los Angeles, CA
Background: Results from the ChronEx RCT, assessing a novel bromelain-based enzymatic debridement (BBD) in the treatment of chronic venous leg ulcers (VLU) were published previously. BBD was superior to hydrogel placebo and non-surgical standard of care (NSSOC), in complete debridement (CD) and complete granulation (CG), key components of wound bed preparation (WBP). One of the NSSOC used in the study was collagenase Santyl® Ointment, approved in the US for debridement of chronic dermal ulcer.

Post-hoc analyses assess the efficacy of BBD vs. Santyl in VLU in ChronEx.

Methods: In ChronEx patients with chronic VLU were randomized (3:2:2 ratio) to daily treatment with BBD, placebo, or NSSOC, for up to 2 weeks or until reaching CD and then followed-up weekly with NSSOC for 12 weeks. NSSOC included Santyl, hydrogels, medical grade honey, and non-active dressings. Surgical or mechanical debridement were not allowed.

Post-hoc analyses assessed incidence and time to CD, CG and WBP in patients treated with BBD compared to Santyl, placebo, and NSSOC excluding Santyl (NSSOCES). WBP was defined as CD and CG, both assessed clinically. Log-rank test was used to compare survival distributions and Fisher Exact test to compare incidences.

Summary of results: Of the 119 patients randomized, 46 were treated with BBD, 43 with placebo and 30 with NSSOC. Of the NSSOC arm, 8 patients were treated with Santyl and 22 were treated with NSSOCES. Overall, the average wound size was 15.5 cm² (SD 19.4), 8 patients were treated with Santyl and 22 were treated with BBD, 43 with placebo and 30 with NSSOC. Of the NSSOC arm, 4 patients were treated with Santyl and 22 were treated with NSSOCES. WBP was defined as CD and CG, both assessed clinically. Log-rank test was used to compare survival distributions and Fisher Exact test to compare incidences.

Summary of results: Of the 119 patients randomized, 46 were treated with BBD, 43 with placebo and 30 with NSSOC. Of the NSSOC arm, 8 patients were treated with Santyl and 22 were treated with NSSOCES. Overall, the average wound size was 15.5 cm² (SD 19.4), average non-viable tissue 73.2% (SD 15.2) and average wound age 31.2 weeks (SD 23.9). Baseline characteristics were comparable across all groups.

Median time to complete debridement (95% CI) was 9 days (5-15 days) for BBD vs. not achieved for Santyl (22-NA, P = 0.023), 63 (21-93) and 44 days (21-67) on placebo and NSSOCES respectively.

Incidence of CD (95% CI) during the two days daily treatment was 63.0% (47.5-76.8) for BBD vs. 0% for Santyl (p = 0.001), 30.2% (17.2-46.1) and 18.2% (5.2-40.3) for placebo and NSSOCES respectively.

Median time to CG as well as to WBP (95% CI) was 11 days (7-50 days) for BBD vs. not achieved for Santyl (22-NA, P = 0.014), 85 (24-99) and 61 days (30-85) on placebo and NSSOCES respectively.

Incidence of WBP (95% CI) during the study was 78.3% (63.6-89.1) on BBD vs. 37.5% on Santyl (8.5-75.5, P = 0.003), 60.5% (44.4-75.0), and 68.2% (45.1-86.1) on placebo and NSSOCES respectively.

Conclusions: Debridement of non-viable tissue and promotion of a well vascularized granulation tissue are key components of WBP, that can support secondary healing or facilitate the effectiveness of other advanced measures. Analysis from the ChronEx RCT demonstrates a clinically meaningful and statistically significant reduction in time to CD, CG and WBP and increased incidence of WBP in VLU patients treated with BBD compared to those treated with Santyl.

Background: Diabetic foot ulceration (DFU) is a complex complication of diabetes, and its pathogenesis remains elusive. Drawing on the success of a strain-programmed patch in expediting wound closure, we conducted a groundbreaking single-cell analysis of diabetic Yucatan minipig skin injuries to unravel the intricate mechanisms of diabetic wound healing at distinct time points.

Methods: In a longitudinal study, we examined various time points (3, 7, 14, and 28 days post-wounding) in Alloxan-induced diabetic swine treated with either Tegaderm, a non-strain patch formulation, or our improved strain-programmable patch formulation. Three wounds per group per timepoint underwent mechanical and enzymatic digestion, generating highly viable single-cell suspensions. Subsequently, we performed detailed single-cell analyses to unveil the molecular landscape of diabetic porcine wound healing.

Results: Sequencing 280,000 cells enabled the identification of expected cell types, including keratinocytes, fibroblasts, endothelial cells, smooth muscle cells, myeloid, and lymphoid cells. We identified several separate subsets of fibroblasts, which are transcriptionally and functionally distinct and change in abundance along the time and treatment axes, with some changes reaching statistical significance. Through differential expression and pathway analyses, we deeply characterize the fibroblast subtypes uncover their functional significance, and reveal, through cell-cell communication analysis, extensive inflammatory pathway activation on Day 7, consistent with an enhanced inflammatory wound healing process. Finally, through trajectory analysis, we propose a model of fibroblast differentiation for each subtype. In addition, leveraging our diabetic foot ulcer dataset and comparing it against mouse wounding studies, we highlight the porcine model’s superior resemblance to human skin wounds.

Conclusion: Our study unveils distinct fibroblast subsets in diabetic porcine wounds undergoing transcriptional and functional changes over time and treatments. Porcine wound cells closely resemble human wounded tissue in both cellular proportions and activated pathways. This comprehensive understanding of diabetic wound healing mechanisms opens avenues for targeted interventions and emphasizes the translational relevance of the porcine model in advancing diabetic wound research.
L3: Biological Dressings and Matrices

L3.01 | Resolution of Bioburden and Wound Closure in a Porcine Full Thickness Wound Model

Justin Avery1, Joel Gil2, Kelly Kimerling1, Stephen Davis2, Katie Mowry1
1Research & Development, Organogenesis, Birmingham, AL; 2Department of Dermatology and Cutaneous Surgery, University of Miami, Miami, FL

Collagen matrices have been shown to provide a target for aberrant proteolytic activity in a chronic wound environment. Combining these matrices with antimicrobials such as silver or polyhexamethylene biguanide (PHMB) has demonstrated both in vitro and in vivo to prevent the reformulation of biofilm. However, a broader understanding of wounds treated with these devices is limited. We designed a porcine full thickness wound model infected with meticillin-resistant Staphylococcus aureus (MRSA) to model the clinical environment of chronic wounds. Evaluation of the impact of a native cross-linked collagen matrix with PHMB (PCMP™) on biofilm reformation, wound closure, and gene expression with STRING and Gene Ontology (GO) term assessment over the course of treatment was performed.

4cm x 4cm x 3mm full thickness wounds were generated on the backs of pigs (n=3), infected with MRSA (USA300), and allowed to form a biofilm for 72 hours. After biofilm formation, wounds were sharply debrided to model standard of care and subsequently PCMP was applied. PCMP was changed every 5 days, at which point wounds were assessed at Days -3 (unwounded baseline, N=4), 0 (infected and debrided baseline, N=6), 5 (N=2), 10 (N=5), 15 (N=5), and 20 (N=3) post-treatment initiation. Gene expression within the wound bed was assessed using RT2 Profiler™ PCR Arrays (Pig Wound Healing, Qia-gen). Genes that were statistically different from unwounded skin were used for STRING analysis with Markov Cluster Algorithm (MCL) inflation parameter of 3. GO terms from these clusters were then plugged into “reduce and visualize gene ontology” (REVIGO) to determine high level GO terms related to various phases of the wound healing process.

Compared to unwounded skin, two pathways were identified: macrophage recruitment/activation and ECM remodeling. We observed statistically increased expression of genes associated with monocyte/macrophages recruitment (CCL2), activation (CD40LG), and subsequent response (IL-1α, IL-1β, IL-10, and TNF), which trended back towards baseline as the wounds healed and bioburden was eliminated. We also observed statistically greater expression of collagenase (MMP1), gelatinase (MMP9), and stromelysin (MMP3) in the wound that resolved as the wound healed. REVIGO analysis found GO terms associated with normal wound repair, starting with inflammation that progressed towards cellular proliferation and migration and ECM remodeling as wounds closed.

Together, these findings highlight that sharp debridement followed by a native cross-linked collagen matrix with PHMB helped prevent biofilm reformation and supported normal wound healing, while the changes in gene expression over the course of closure were characterized.

^PuraPly® AM, Organogenesis, Canton, MA

L3.02 | Efficacy and Safety Of Dressing Containing Novel Antimicrobial Peptide For Managing Wound Biofilm

Jennifer Neff, Ryan Cummings, Danir Bayramov
Allvivo Vascular, Inc., Lake Forest, CA

Purpose: Biofilm colonization, delayed healing and drug resistance remain important challenges in wound care. The purpose of this work was to evaluate a wound treatment comprising a novel broad-spectrum antimicrobial peptide (ASP-2) formulated in a biodegradable chitosan sponge (Gatekeeper™) for activity against multi-drug resistant bacterial biofilm, to assess the propensity for ASP-2 resistance to evolve with exposure, and to evaluate product safety.

Methods: Efficacy was evaluated using an ex vivo porcine skin biofilm model. Disks of porcine skin were incubated in bacterial culture (10^6 CFU/mL of Methicillin resistant Staphylococcus aureus, USA 300 ATCC BAA-1717 (MRSA) or Pseudomonas aeruginosa ATCC 15692 (P. aeruginosa)) for 72h to develop biofilm. Skin disks were then treated once with Gatekeeper™ and bacteria CFU remaining on skin were measured after 24, 48, and 72 hours. To evaluate potential for resistance development, MRSA was serially passaged in 0.5 MIC ASP-2 for 30 days. MICs for ASP-2, vancomycin, and mupirocin, were measured daily for ASP-2 exposed cultures with the CLSI M07-A8 microlodination method. The tolerability and toxicokinetics (TK) of ASP-2 and Gatekeeper™ in minipigs were determined following daily administration over 7 days, respectively.

Results: In the ex vivo porcine skin biofilm model, Gatekeeper™ application resulted in a 5.7 log reduction in MRSA CFU/mL by 24 hours with full eradication by 48 hours. P. aeruginosa biofilms experienced 3-4 log reductions in CFU/mL by 24 and 48 hours and were eradicated by 72 hours. All reductions were statistically significant relative to saline controls (ps<0.05, n=3 for all groups). Exposure to subinhibitory concentrations of ASP-2 did not select for resistance in MRSA. There was a 2-fold increase in ASP-2 MICs after the first passage, with no significant increases measured over the remaining 29 days of passaging. Moreover, bacteria exposed to thirty passages of sub-MIC ASP-2 did not display cross-resistance to vancomycin or mupirocin.

In toxicology studies, the no observable adverse effect level of ASP-2 in rats with subcutaneous administration (N=10) was 100 mg/kg (>20X the equivalent human clinical dose). In minipigs, no toxicologically meaningful effects were found with Gatekeeper™ treatment of full thickness wounds over 2.5% body surface area (BSA) (N=2) or abraded skin over 10% BSA (N=2). Systemic exposure to ASP-2 in minipig TK studies was also found to be minimal.

Conclusions: Gatekeeper™ exhibited strong activity against MRSA and P. aeruginosa biofilms and initial toxicology studies indicate a good therapeutic window for its active component, ASP-2. Serial passaging of MRSA in subinhibitory concentrations of ASP-2 did not result in...
emergence of ASP-2 resistance or cross resistance to front line antibiotics tested. Collectively, data presented indicate a promising efficacy and safety profile for Gatekeeper™ in managing wound biofilm.

**L3.03 | Novel Antibiotic-Free Biomimetic Wound Matrix Provides Antimicrobial Protection And Superior Healing**

Bruno F. Caetano, Trudy-Ann Grant, Bishnu P. Joshi, Adam Finzen, Tarak Bhakda, Rebecca Salamone, Manav Mehta, Ana Tellechea

*Gel4Med Inc., Lowell, MA*

**Background:** Pathogenic colonization is a major risk factor for acute and chronic wound complications. Current approaches have serious limitations, and the rise of multidrug-resistant organisms (MDROs) and biofilms further complicates treatment. To address this growing issue, we developed and tested a polypeptide biomimetic wound matrix (BWM) that prevents infection while promoting healing. This self-assembling cationic nanofiber technology was engineered to offer a mechanism of action that evades microbial resistance, a scaffolding matrix with cell attachment sites that encourage tissue regrowth, and wound-conforming properties for tissue void filling.

**Methods:** Antimicrobial efficacy against Gram-positive [*Enterococcus faecium*, *Staphylococcus epidermidis*, *Staphylococcus haemolyticus*, MRSA] and Gram-negative [*Pseudomonas aeruginosa* (PaO1), ESBL *Escherichia coli*, ESBL *Klebsiella pneumoniae*, *Acinetobacter baumannii*] bacteria, as well as fungi [*Candida albicans*, *Aspergillus fumigatus*] was tested by standard time-kill assays. Efficacy against 72 hour-aged biofilms (PaO1 ± MRSA) was assessed *in vitro* and *ex vivo* (porcine skin). MRSA-inoculated murine wounds were treated with BWM and assessed by microbiology at 24 h. BWM mechanical properties were confirmed by rheology and wound bed coverage. In a swine model of full-thickness excisional wounds, BWM healing efficacy was tested vs. silver and collagen gels using the Tissue Analytics platform and histopathology.

**Results:** *In vitro*, BWM demonstrated bactericidal efficacy against ≥6 log10 CFU of PaO1 and MRSA at 5, 10, 15, 30, 60 min, and 24 h (p<0.0001, n=3). Within 24 h, BWM eliminated ≥6 log10 CFU of Gram-positive and Gram-negative clinical isolates, as well as sporulating and non-sporulating fungal pathogens. Notably, BWM showed superior efficacy against PaO1 and MRSA biofilms when compared to marketed antimicrobial gels (p<0.0001, n=6), while a single application eradicated mature PaO1 biofilms in pig skin explants by 24 h (n=3). Rapid bioburden reduction was confirmed in MRSA-inoculated murine full-thickness wounds (p<0.02, n=10). In a trypsin proteolysis assay, matrix stability was demonstrated for up to 14 days. BWM-treated swine full-thickness excisional wounds showed superior closure rates (96%, n=5) vs. collagen gel and silver gel (p=0.01) and reduced inflammation. BWM singularly achieved complete re-epithelialization with healthy granulation tissue repletion by day 14.

**Conclusions:** BWM demonstrates potent broad-spectrum antimicrobial activity against MDROs and biofilms. With controlled biodegradation in a highly proteolytic environment, BWM shows a wound healing profile superior to commercial silver and collagen dressings, including greater closure, increased re-epithelialization, granulation tissue formation, and reduced inflammation. Together, the data supports BWM potential to overcome the current challenges in managing complex and infected wounds.

**L3.04 | Treating Diabetic Fibroblasts Through Tunable Hyaluronan-Binding Silk Fibroin Therapeutic Hydrogels**

Amelia Huffer, Tugba Ozdemir

*Nanoscience and Biomedical Engineering, South Dakota School of Mines and Technology, Rapid City, SD*

Granulation tissue formation in diabetic wounds is predominantly controlled by proliferation and extracellular matrix (ECM) deposition of diabetic fibroblasts (DFs). Accumulating evidence shows the proliferation and ECM deposition of DFs are significantly different than their normal counterparts. More specifically DFs are known to deposit altered ECM such as lower levels of Hyaluronan (HA) and higher crosslinking of collagens. The altered ECM in diabetic wounds results in altered mechanical properties and accumulating evidence shows surrounding mechanical properties of the matrix have an impact on cell behavior. We hypothesized that wound dressings designed towards use in diabetic wounds would address matrix mechanics alongside matrix composition. We designed a tunable stiffness Silk Fibroin (SF) hydrogel matrix with an ability to attract and retain endogenous HA to accommodate for altered stiffness and compromised HA levels in these pathologies. The SF hydrogels will be grafted with a novel HA binding peptide (HABP) to aid in the attraction of these HA. Our goal is to create a therapeutic biomaterial platform to treat diabetic fibroblasts into a healthy phenotype. *Creation of silk hydrogels.* Mechanical mixing and lyophilization were used to develop the 3D SF hydrogels of varying stiffnesses. Silk binding peptide (SBP) motifs were used to aid in the grafting of the HABP to the SF hydrogels. SEM imaging, swelling ratio, and DMA were used to physically characterize the hydrogels. A carbazole assay was used to measure the endogenous HA binding capacity. *Diabetic fibroblast physiology.* Metabolic activity, proliferation, and cell morphology assays were used to measure the effect of the SF hydrogel HA binding capacity on dhDF. To evaluate the role of HABP directed HA deposition on dhDF physiology we performed a PCR array targeting wound healing associated genes. We found softer substrate stiffness caused the dhDF to behave more like the hDF in both metabolic activity and with the cell morphology. There was a 15.6% decrease in the aspect ratio and a 16.4% increase in circularity for the dhDF when placed on a softer surface. This data was indicative that the dhDF were beginning to exhibit more fibroblast like behaviors on a softer surface. In light of these results, we developed microporous SF hydrogels and current studies undergoing to incorporate HABPs through silk binding peptide sequences. The swelling ratio of these SF hydrogels are 26.04 ± 1.77% and the pore size is 62.056 ± 31.985 mm. Based on our findings we anticipate that the SF hydrogels with HABP to will retain endogenous
HA and cause therapeutic changes in the physiology of the dhDF similar to our stiffness studies.

**L3.05 | Finite Element Analysis, Preclinical, And Proteomic Assessment Of A Novel 7-Day Extended Wear Peel And Place Negative Pressure Wound Therapy Dressing**

Diwi Allen1, Samantha A. Mann1, Balakrishna Haridas2, Brenda Marchand1, Marisa Schmidt1, Kris Kieswetter1
1Medical Solutions Division, 3M Company, San Antonio, TX; 2Device & Implant Innovations, College Station, TX

**Problem:** Negative pressure wound therapy (NPWT) with reticulated open cell foam (ROCF) necessitates frequent dressing changes as tissue ingrowth may occur if left in place for greater than 72 hours.

**Objective:** These studies evaluated key wound healing-associated characteristics of a novel NPWT peel and place dressing designed for extended wear use.

**Methods:** Finite element analysis (FEA) was conducted using computer simulation to evaluate the effects of NPWT on tissue deformations produced by the peel and place dressing. Wound models were developed, with clinical input, incorporating dimensional specifications and material properties for the relevant tissue layers (epidermis, dermis, subcutaneous fat, muscle, and bone). An additional preclinical assessment was conducted using a swine model with full-thickness, excisional paraspinal wounds in 11 animals, and continuous -125mmHg NPWT for 7 days. The study was designed to assess long term wear; therefore, no dressing changes were performed throughout the 7-day study. The wounds were dressed with either peel and place dressing or ROCF. Biopsies were collected for protein extraction and tissues excised for histology at study termination. The extracted protein was assessed using a multiplex immunoassay to quantify wound healing biomarkers, and histologic morphology was used to measure granulation tissue thickness. The study was approved by an Institutional Animal Care and Use Committee (IACUC) and animal care complied with all applicable national and local regulations.

**Results:** FEA revealed homogenous tissue displacements, uniform tissue tensile strains, and notable volume of tissue engagement. Preclinical results demonstrated significantly more granulation tissue than ROCF (p<0.05) with tissue ingrowth being limited to only the ROCF treatment (p<0.0001). Proteomic analysis of wound healing-associated cytokines/chemokines and heparin-binding endothelial like growth factor (HB-EGF) demonstrated elevated levels of interleukins (IL)-1α, IL-1β, IL-1ra, IL-8, IL-12, and HB-EGF in the peel and place dressing treatment as compared to ROCF (p<0.05).

**Conclusion:** Greater mechanical stimulation generated by the peel and place dressing, as illustrated by FEA, is likely to have contributed to cell signal transduction, promoting elevated levels of wound healing biomarkers. The elevated presence of these critical biomarkers, in turn, supported greater granulation tissue formation promoted by the peel and place dressing. This outcome, along with mitigated tissue ingrowth, support the effectiveness of the peel and place NPWT dressing for 7-day extended-wear.

3M™ V.A.C.® Therapy; 3M™ V.A.C.® Granufoam™ Dressing; Peel and Place Dressing (3M, San Antonio, TX)

**L3.06 | Wound Histology Through Virtual Staining Using Generative Adversarial Networks**

Malavika Nidhi, Jake Jones, Alan Woessner, Kyle Quinn
Biomedical Engineering, University of Arkansas, Fayetteville, AR

Hematoxylin and Eosin (H&E) staining is the gold standard for visualizing wound microstructural features. However, traditional biopsy, sectioning, and staining is destructive, variable, and cannot capture dynamic events. Label-free multiphoton microscopy (MPM) allows for non-invasive in vivo 3D visualization of tissue microstructure using the endogenous fluorescence of cellular metabolic cofactors as well as second harmonic generation of collagen. However, MPM-generated images are unfamiliar to pathologists, clinicians, and biologists accustomed to interpreting H&E images. Bridging this familiarity gap is crucial for integrating advanced imaging into established diagnostic practices.

Generative Adversarial Networks (GANs) are deep learning models used for style transfer and image-to-image translation tasks. Typical GANs consist of a Generator network that can create or modify images and a Discriminator network that tries to distinguish real and computer-generated images, guided by loss probabilities. A Cycle GAN is a specific GAN architecture useful for unpaired image translation and employs two Generators (along with two adversarial Discriminators) to translate images between two domains. The objective of this project was to develop a Cycle GAN capable of translating unstained MPM images of wounds to resemble H&E sections.

A Cycle GAN architecture was written in Python utilizing PyTorch. The Generator networks learned to transform MPM images of unstained tissue to resemble H&E stained sections and vice versa, while the Discriminator networks learned to tell the difference between the generated images and real H&E or MPM images. All the networks learned from mistakes of the Discriminators during training, and the Generators also learned from how similar computer-generated images look when transferred from one image domain and back. Our Cycle GAN was trained over 80 epochs on diverse MPM and H&E image datasets of excisional skin wounds.

Visual comparisons between virtually- and chemically-stained images revealed promising outcomes, with the Generator preserving tissue morphology at a high resolution, while accurately staining epidermis and follicles purple and collagen pink. Most measures of loss (i.e. network error) decreased throughout training, with the noteworthy exception of the Discriminator loss. This suggests a successful outcome, indicating the Generators created convincing virtual staining that the Discriminators ultimately could not discern from real chemically-stained sections. This study demonstrates the potential of virtual staining via Cycle GAN to bridge the familiarity gap between...
MPM and traditional H&E staining. It provides a framework for obtaining high-resolution H&E images of wounds from in vivo imaging without the need for a tissue biopsy, sectioning, or staining.

**L4.01 | Easing The Pressure In Wound Therapy: Evaluating ChatGPT's Management And Treatment Of Wounds**

Daniel Najafal, Timothy W. King

**Background:** ChatGPT, a large language model utilizing generative artificial intelligence (AI), is the fastest-growing consumer application. As an emerging tool that is convenient, it has been accessed for medical advice and applied to healthcare with potential in providing patient education and guidance to practitioners in the management of cases. Wound care is a critical and common medical concern. This study comprehensively evaluates ChatGPT’s capacity to answer users’ questions regarding wound care, efficacy in providing evidence-based recommendations, and effectiveness in offering treatment strategies extrapolated from images from performed cases.

**Methods:** ChatGPT (GPT-3.5) was prompted using open-ended frequently asked questions that were web scraped and adapted from institutional medical websites. Common Questions About Wound Care from the American Academy of Family Physicians (AAFP) were also used. Agreement metrics between modalities were calculated and overlapping keywords were compared. The chatbot's references were scrutinized. Images from published open access case reports pertaining to wound care were provided to GPT-4 to determine its assessment and recommendations for treatment support.

**Results:** Across 6 institutions and the prompts (22 questions) provided, 22 (100%) answers were in agreement with the chatbot’s response and 55/56 (98%) keywords were overlapping. The request for references retrieved 22 publications with 21 (95%) being legitimate (9 were open access). From the AAFP prompts, 8 (100%) agreed and 23 (74%) keywords were overlapping. A total of 10 images from 2 female and 4 male patients were provided from 5 published case reports with a mean (SD) age of 47 (12) years. GPT-4 demonstrated competence with initially assessing and providing global recommendations for hypothetical treatment approaches as well as highlighting negative pressure wound therapy settings, wound healing stages, and possible flap coverage, among other factors, but in the majority of cases diverted from offering exact specifications for management of the patient when questions were open ended. Binary prompting or offering a range of treatment options in conjunction with providing patient information, wound characteristics, and medical history was a superior approach.

**Conclusion:** ChatGPT demonstrated a comprehensive understanding of wound care and management. Although prone to possible hallucinations and varying performance based on prompting scheme, ChatGPT and its successor, GPT-4, have immense potential in serving as an adjunct to patients and providers. Fine tuning a chatbot using relevant articles and cases may be a valuable investment for more profound strides in AI-based involvement for wound care.

**L4.02 | DNA Methylation Profiling for the Prediction of Recurrences and Prognosis in Patients with Diabetic Foot Ulcers**

Sik Namgoong

**Plastic Surgery, Stanford University, Irvine, CA**

**Background:** Diabetic foot ulcers (DFUs) are recalcitrant to healing. However, the molecular mechanism causing this dysfunction is not fully understood. DNA methylation profiles change during the proliferation, differentiation, and development of an organism, resulting in tissue or disease identification. To elucidate the biomarkers for DFU prognosis, we hypothesized that differences in DNA methylation patterns could provide important therapeutic targets in the treatment of DFUs.

**Methods:** We collected 48 blood samples from 36 DFU patients treated at Korea University Guro Hospital from October 2019 to November 2021. The Illumina MethylationEPIC (850k) DNA methylation microarray was used to determine the pattern between differentially methylated regions (DMRs) in DFU patients with good or poor prognoses. We then selected and visualized the DMRs in the form of heatmaps, and enriched terms associated with these DMRs were identified. By using the DMR list in two processes, Kyoto Gene and Genome Encyclopedia (KEGG) and gene ontology (GO) analysis, gene-concept network, GSEA, and decision tree were performed.

**Results:** In total, 92 DMRs and 108 DMRs (Log2 fold change > 0.1 and P < 0.03) were hypermethylated and hypomethylated, respectively. In the good prognosis sample, 69 and 156 DMRs were hypermethylated and hypomethylated, respectively. In the KEGG analysis, the MAPK signaling pathway was commonly detected as the highest pathway. In the decision tree, MORN1 hypomethylation and NCOR2 hypermethylation were crucial classifiers by recurrence.

**Conclusion:** Collectively, MORN1 and NCOR2 genes may be used as biomarkers for predicting the recurrences and prognosis in DFU patients. In DFUs, the clues of recurrence and prognosis prediction may be provided through DMRs and the molecular mechanisms related to inflammation.

**L4.03 | Racial Disparities In Incidence Of And Access To Hypertrophic And Keloid Scar Management**

Stuti P. Garg, Krish Shah, Robert Galiano

**Case Western Reserve University School of Medicine, Cleveland, OH; Northwestern University Feinberg School of Medicine, Chicago, IL**

**ABSTRACTS**
**Background:** Incidence of hypertrophic and keloid scars is influenced by inherent skin attributes such as skin color. Both are clinically challenging to forecast and manage. This study aims to understand differences in occurrence and access to treatment to normalize skin color as consideration of scar management following surgery or trauma in order to achieve optimal results.

**Methods:** Using the All of Us Research Program’s Data and Research Center (DRC), the incidence of hypertrophic and keloids scars segmented by race was retrospectively analyzed. Proportions of scars for Black and White self-identifying patients were compared to the rates of access to treatment, defined as either topical or revision protocol to address scars by race. Chi-squared test and Mann–Whitney U test were used to determine statistical significance between groups.

**Results:** 10,910 total scars were included. Hypertrophic and keloid scars represented 16.0% and 12.6% of these scars across all races. Scars in Black patients represented 31.9% of hypertrophic scars and 34.5% of keloids while overall scars from Black patients only represented 17.4% of the total (p<0.05). Black patients only represented 20.2% of all scars treated topically and 20.0% of scars treated with a revision protocol. There was a statistically significant difference between the proportion of hypertrophic and keloid scars represented by Black patients and the proportion of scars treated (p<0.05).

**Conclusion:** Black patients have a disproportionately lower rate of access to treatment as compared to the rate of incidence of hypertrophic and keloid scars. As Black patients are more likely to have worse perceptions and lower probability of improvement for these scar types, it is important to increase access to treatment options. This study highlights the need to address patient race when considering the healing process of wounds.

---

**L4.04 | Evaluating TEWL as a Predictive Marker for Reulceration in Healed Chronic Diabetic Foot Ulcers**

Rawlings E. Lyle1, Pallas Lim2, Mirabel Dafinone2, Sara Dahle2, Rivkah Isseroff2

1School of Medicine, University of California, Davis, Sacramento, CA; 2Dermatology Section, VA Northern California Health Care System, Mather, CA; 3Podiatry Section, VA Northern California Health Care System, Mather, CA; 4Department of Dermatology, University of California, Davis, Sacramento, CA

**Background:** Chronic diabetic foot ulcers (DFUs) present a significant healthcare challenge due to their high recurrence rate and associated morbidity. Identifying reliable predictive markers for ulcer reulceration is critical for improving patient outcomes. This study investigates the role of transepidermal water loss (TEWL) as such a marker. TEWL’s potential relevance stems from its reflection of skin barrier function, which is crucial in wound healing and prevention of complications.

**Methods:** Nineteen patients, averaging 69 years of age (±6.4 years), including 50% with a smoking history, were enrolled. These individuals had an average Charlson comorbidity index of 5.7 (±1.5) and a mean diabetes duration of 21 years (±6.7 years). Wound chronicity varied from 8 to 53 weeks with a mean of 20.8 weeks. TEWL was measured using a Vapometer™ device at the healed wound site, 5.0 cm from the wound site, the identical site on the contralateral leg, and 5.0 cm from the contralateral site at three to four monthly visits. Reulceration occurred at a mean of 78.7 weeks (±60.6 weeks) after initial healing, and the time from the last TEWL reading to reulceration ranged from 4 to 146 weeks.

**Results:** A significant difference was observed in TEWL values at the healed ulcer site between patients whose ulcers remained healed and those who re-ulcerated within 3 months to 3 years post-study. Patients who did not re-ulcerate had a mean TEWL at the healed wound site of 22.85, compared to 27.04 in patients who subsequently re-ulcerated (p<0.001). The difference in TEWL between the wound and contralateral sites was 4.46 in patients who did not re-ulcerate and 7.61 in those who re-ulcerated (p=0.005). Significance and analysis were determined by T-tests and generalized linear modeling.

**Conclusions:** The results indicate that TEWL, especially the previously unrecognized variance between the wound and contralateral sites, may serve as a reliable indicator for predicting the risk of re-ulceration in patients with healed DFUs. However, the generalizability of these findings is limited due to the small sample size, suggesting a need for further research with larger cohorts. Future studies should aim to establish TEWL norms in healthy individuals for better comparative analysis and the development of effective preventative strategies for diabetic patients, considering the high 5-year mortality rate associated with DFUs.

---

**L4.05 | Discarded Wound Dressings: An Untapped Source Of Predictive And Monitoring Biomarkers Of Multiple Types Of Wounds**

Victoria Soto1, Sujad Younis2, Natasa Strbo2, Matthew Hardy2, Jason Levine4, Monica Perez4, Juan O. Bravo1, Hadar Lev-Tov1, Robert S. Kirsner1, Ivan Jozic1

1Dermatology, University of Miami School of Medicine, Miami, FL; 2Microbiology and Immunology, University of Miami Miller School of Medicine, Miami, FL; 3Miami VA Medical Center, Miami, FL; 4Division of Endocrinology, Metabolism and Diabetes, University of Miami Miller School of Medicine, Miami, FL

**Purpose:** The purpose of our study was to utilize discarded wound dressings in order to identify potential predictive and diagnostic biomarkers of different wound types by the proteomic analysis of cells and exosomes captured from wound exudate.

**Methods:** We solubilized biomaterial from discarded wound dressings from patients with pyoderma gangrenosum, diabetic foot ulcer, venous leg ulcer and acute wounds left to heal by secondary intention. Patients were followed weekly for 4 weeks and change in wound size measured by EKARE inSight imaging equipment at each visit, at which point the wound dressing was collected. Cells from each dressing were then isolated, number of viable cells quantified and
Detection of Predictive Human Genetic Markers Of The Wound Microbiome

Rebecca Gabrilská1, Khalid Omeir2, Ashley Noe2, Jacob Ancira2, Clint Miller3, Craig Tipton2, Kendra Rumbaugh1, Joseph Wolcott3, Nicole Phillips4, Caleb Phillips2
1Surgery, Texas Tech University Health Sciences Center, Lubbock, TX; 2Biological Sciences, Texas Tech University, Lubbock, TX; 3Southwest Regional Wound Care Center, Lubbock, TX; 4Microbiology, Immunology & Genetics, University of North Texas Health Sciences Center, Fort Worth, TX

Background: Chronic wounds are a burden to millions of patients and healthcare providers worldwide. With rising incidence and prevalence, there is an urgent need to address these non-healing wounds with novel approaches. Impaired wound healing has been shown to be associated with the wound microbiota, as multiple bacterial species are known to contribute to infection and delays in closure. Whether there is an urgent need to address these non-healing wounds with novel approaches. Impaired wound healing has been shown to be associated with the wound microbiota, as multiple bacterial species are known to contribute to infection and delays in closure. Microbial communities are shaped by host genetics is less understood. Microbiome genome wide association studies (mbGWAS) can provide insight into host genetic factors that may influence bacterial community structure. Previous work reports that the alpha diversity of the chronic wound microbiome is significantly associated with specific human genomic loci and healing.

Methods: To compare wound microbiomes to human genomes, we performed a two-stage mbGWAS using a cohort of 458 patients. Briefly, patients with lower extremity, non-healing wounds were consented from the Southwest Regional Wound Care Center in Lubbock, TX. Buccal swabs and wound samples were collected then genotyped and sequenced for bacterial taxa, respectively. After quality control and genome imputation, mbGWAS was then performed to test association of the relative abundances of multiple wound-relevant bacteria with patient genotype.

Results: We identified bacterial taxa that are repeatedly significantly associated with single nucleotide polymorphisms (SNPs) in the host genome. The significance threshold was set at the Bonferroni adjusted p value of <0.05. Hundreds of unique SNPs across multiple unique bacterial species were repeatedly associated, and these species included both common and uncommon members of wound microbiomes.

Conclusions: Using an mbGWAS design, we reveal human genomic markers that significantly associate to specific bacterial taxa, suggesting a genetic predisposition to colonization or infection by members of the wound microbiome. Future studies will involve translating these mbGWAS findings to functionally validate likely causal SNPs. Identification of correlated biomarkers may provide new mechanistic insight into microbe-host interactions and may serve as predictive risk factors to guide personalized management for chronic wound patients.

WHS SESSION O: Rapid Fire Poster Presentations

Impact of Extracellular Matrix Graft Composition on Degradation Dynamics and Scaffold Functionality

Katrina Harmon, Miranda Burnette, Justin Avery, Kelly Kinnerling, Katie Mowry
Research & Development, Organogenesis, Birmingham, AL

Extracellular matrix (ECM) grafts are emerging as a promising treatment option for chronic wounds due to their scaffold properties. Here, two porcine small intestinal submucosa-derived cross-linked collagen matrices with PHMB (PCMP,2layers; PCMP-XT,5layers) were compared to ovine forestomach matrix (OFM,1layer) derived from propria submucosa and assessed for their durability in a simulated wound environment and functionality as a scaffold by their ability to inhibit proteases and support cell attachment and proliferation. Matrices were evaluated for structure using scanning electron microscopy (SEM) and histology, ability to modulate matrix metalloproteinases (MMPs) using fluorometric assays, and graft durability using an in vitro degradation model comprised of simulated wound fluid plus collagenases type I and II (SWF+.). Throughout degradation, scaffold...
functionality was assessed using an in vitro primary human dermal fibroblast cell attachment model.

Grafts differed in matrix structure, composition, and thickness. SEM demonstrated a more fibrous structure in OFM and PCMP-XT, which was confirmed following histological evaluation. When evaluating tissue thickness, PCMP-XT was significantly thicker than PCMP, while PCMP and OFM were comparable. All matrices resulted in inhibition of collagenases, gelatinases, and stromelysins, but PCMP and PCMP-XT were overall more inhibitory compared to OFM. In an in vitro SWF + degradation model, OFM degraded rapidly (<3 hours) and was therefore omitted from further analysis. Both PCMP and PCMP-XT significantly degraded in the SWF + model within 7 days (p<0.0001), with PCMP having a significantly higher degradation rate (p<0.05). To assess scaffold functionality, intact (non-degraded) and degraded grafts were seeded with primary human fibroblasts and evaluated for attachment and proliferation over 7 days. Cells readily attached to both matrices. Cells seeded on PCMP resulted in significant proliferation on days 3 and 7 (p<0.01), while cells seeded on PCMP-XT demonstrated significant proliferation at day 7 (p<0.05). To assess the ability of matrices to support cell attachment through degradation, 3- and 5-day SWF + degraded scaffolds were evaluated for cell proliferation for 7 days. PCMP and PCMP-XT exhibited robust cell attachment on 3- and 5-day degraded scaffolds, with significant proliferation on both by day 7. Interestingly, 5-day degraded PCMP resulted in overall more proliferation compared to 3-day degraded samples on day 7 (p<0.01), while proliferation was comparable for both 3- and 5-day degraded PCMP-XT groups (p<0.0001).

Together, these findings demonstrate that differing compositions of ECM grafts result in varying MMP inhibition and durability. Throughout degradation, both PCMP and PCMP-XT demonstrated a maintenance of scaffold properties by supporting fibroblast attachment and proliferation.

Puraply® AM; Puraply® AM-XT, Organogenesis, Canton, MA

Endoform, Aria Biosurgery, San Diego, CA

Briefly, NHDFneos and NHLFs were embedded in a type I collagen gel and seeded into μTug devices composed of wells with micropillars to form 3D microtissues. 24 hours after the tissues formed, a Nd:Yag Laser was used to create full-thickness wounds in the center of the tissue. Both NHDFneo and NHLF tissues were treated with contractility or adhesion modulators before injury and wound closure dynamics were assessed for 24 hours post-injury using time-lapse microscopy.

Traction force microscopy was performed on the individual cell types seeded on type I collagen coated 5 kPa polyacrylamide gels under different treatment conditions using a Nikon-Eclipse-Ti microscope. Immunofluorescent staining against phosphorylated-paxillin was performed to visualize and quantify size of focal adhesions. In some experiments, staining against cellular fibronectin was performed to visualize provisional matrix assembly of fibers in tissues 24 hours post-injury.

At baseline, NHDFneos healed the wounds in the tissues within 24 hours post-injury, while NHLF tissues failed to close the wounds. On 2D substrates, NHDFneos exhibited lower contractility and smaller adhesions compared to NHLFs. Treatment with manganese (Mn) increased focal adhesion size and contractility of NHDFneos as well as inability to heal full-thickness wounds in tissues. Mn-treated NHDFneo tissues revealed clustering of cellular fibronectin with little to no fibrillogenesis throughout the tissue post-injury, while untreated tissues assembled fibronectin fibers throughout the remodeled tissue post-injury. Conversely, treatment of NHLFs with a low dose FC11, a FAK inhibitor, lowered contractility and the size of focal adhesions to comparable levels as untreated NHDFneos. NHLF tissues treated with FC11 healed within 24 hours post-injury while untreated tissues failed to heal (N = 3, n = 20 tissues per group). Interestingly, NHLFs treated with blebbistatin and Y-27632, a myosin II and ROCK inhibitor respectively, exhibited lower contractility, but continued to fail healing tissues. Together, our data support the hypothesis that the balance between contractility and adhesion levels modulates wound closure in our microtissue system, which may inform new mechanotherapeutic strategies for treating impaired wound healing.

0.2 | The Balance Between Cell Contractility And Adhesion Modulates Provisional Matrix Assembly During Wound Closure

Emily Davis, Marina Uroz, Christopher Chen, Jeroen Eyckmans
Biomedical Engineering, Boston University, Brookline, MA

Upon injury of fibrous tissues, resident fibroblasts migrate into the wound bed, depositing a fibrous provisional matrix and pull the margins of the wound closer to accelerate wound closure. While cellular contractility and cell adhesion are two processes regulating wound healing, it remains unclear how these processes coordinate provisional matrix assembly during stromal closure. To address this question, we manipulated cell contractility and cell-matrix adhesion of Normal Human Dermal Fibroblasts Neonatal (NHDFneos) and Normal Human Lung Fibroblasts (NHLFs) and assessed wound closure in a 3D microtissue model of stromal wound healing.

0.3 | Mechano-Immuno-Fibrotic Wound Healing Pathways Regulate The Cellular And Molecular Ecology Of Foreign Body Response

Andrew Hostler, William Hahn, Jenne Stensland, Katharina Fischer, Abdelrahman Alsharif, Maria Gracia Mora Pinos, Hudson C. Kussie, Autumn Lester, Fidel Saenz, Jonathan P. Yasmeh, Eamonn McKenna, maisam Jafri, Maia Granoski, Jose Vasquez, Amelia B. Knochel, Aaron Mason, Kellen Chen, Geoffrey C. Gurtner
Surgery, University of Arizona College of Medicine -Tucson, Tucson, AZ

Background: Implantable biomedical devices have revolutionized medicine, with >70 million device implantations annually that benefit millions of patients. ~30% of devices will undergo premature failure during their lifetime, primarily due to an immune-mediated physiologic reaction known as the foreign body response (FBR).
Biophysiocochemical incompatibilities between host tissues and device biomaterials, regulated through immune signaling, activates the wound healing pathway resulting in fibrosis and collagenous tissue encapsulation of the device. Herein, we characterized the cellular and molecular ecology of human FBR capsule tissue.

**Methods:** Breast implant FBR tissue was collected with an IRB protocol from patients (n=14) undergoing standard of care surgical exploration of biomedical devices. Baker capsular contracture grading (B1: n=5; B2: n=3; B3: n=2; B4: n=4) was evaluated. Histological staining and analysis using hematoxylin and eosin (H&E), Masson’s trichrome, and picrosirius red was performed to assess tissue morphology, collagen deposition, and collagen architecture. Immunofluorescent staining and analysis was performed using CD68 (macrophage marker), αSMA (myofibroblast marker), and FAK (mechanical signaling marker).

**Results:** Severe FBR, Baker grades 3 & 4 (B3/B4), demonstrated significantly increased capsule thickness on H&E staining compared to mild FBR B1/B2 (p=0.0006). Analysing collagen architecture with software algorithms CT-FIRE and CurveAlign, we found severe FBR significantly increased collagen fiber length compared to mild FBR (p=0.0408), while also creating a trend of increased collagen fiber angle and density (p=0.1920 and p=0.1774) and decreased mean distance (p=0.1093).

Severe FBR caused an increase in CD68+ (macrophage) and αSMA (myofibroblast) layering within the FBR capsule, shown by significantly increased co-localized expression of CD68 and αSMA (p<0.01) and co-localized CD68 and FAK (p=0.0465). More severe FBR also increased αSMA and immune cell numbers (p<0.009).

**Conclusions:** We uncovered that increased immune-mediate crosstalk between macrophages and myofibroblasts within the FBR capsule correlated with higher degree Baker capsules, characterized by elevated fibrosis and collagen deposition. Interestingly, more severe FBR in humans also caused more colocalized macrophage-myofibroblast cellular layering, as well as elevated mechanoresponsive myeloid cells. Since myeloid cells circulate through the blood stream and home to sites of wound injury, targeting these myeloid cells could lead to new therapies to prevent FBR.

**ABSTRACTS**

### 0.4 | Senescent-Associated Extracellular Matrix Production And Delayed Wound Healing By Senescent Dermal Fibroblasts

Anish Srinivas Vasan, Emma Lejeune, Wilson Wong, Daniel S. Roh, Christopher Chen, Jeroen Eyckmans

1Biomedical Engineering, Boston University, Boston, MA; 2School of Medicine, Boston University, Boston, MA

**Background:** After skin injury, a sub-population of the dermal fibroblasts undergo senescence during the early stages of the healing process. While the transient appearance of senescent cells is required for normal wound healing, the accumulation of persistent senescent cells is associated with delayed and chronic wound healing. The mechanisms by which senescent fibroblasts and their associated extracellular matrix (ECM) impair wound healing are still elusive. Given the role of fibroblasts to produce a provisional matrix during tissue closure, we hypothesize that senescent human dermal fibroblasts produce a senescent-associated ECM that has a distinct composition and tissue architecture compared to non-senescent fibroblasts which delays wound closure.

**Methods:** To address this hypothesis, we leveraged our 3D wound-on-chip microtissue platform to study the effect of senescent cells on stromal tissue repair and ECM architecture. Microfabricated devices consisting of 4 micropillars were cast in a 96-well plate using polydimethylsiloxane (PDMS). We seeded normal or senescent human dermal fibroblasts from neonatal foreskin in a rat-tail collagen type I matrix and allowed them to self-assemble into anchored microtissues. Senescence was induced in the normal fibroblasts through multiple exposures to hydrogen peroxide and confirmed with positive stains for p21, γ-H2AX, and senescent-associated-β-galactosidase. Microtissues were then ablated to create full-thickness wounds using a pulsed nanosecond laser. The provisional ECM was visualized with immunofluorescence labeling and confocal microscopy.

**Results:** We found that microtissues composed of senescent human dermal fibroblasts exhibited significantly delayed wound closure (41 ± 6.27 hours, n=9, N=3) when compared to normal human dermal fibroblasts from neonatal foreskin (26.2 ± 3.53 hours, n=9, N=3). Senescent fibroblasts deposited more fibronectin, collagen type I, collagen type III, and tenascin-C in the provisional matrix than normal fibroblasts. Interestingly, confocal microscopy also revealed that senescent microtissues contained larger, aligned fiber bundles of all four ECM components compared to the fine, mesh-like deposition in normal fibroblast microtissues. Notably, tenascin-C was deposited in small globules in normal fibroblast microtissues, highlighting the stark organizational differences between these fibroblast phenotypes.

**Conclusion:** Together, these data demonstrate that senescent cells produce a senescent-associated matrix with a tissue architecture that is distinct from the ECM produced by non-senescent fibroblasts. This may contribute to the delayed healing observed in senescent microtissues. Our 3D microtissue model provides a robust platform to further study the role of stromal cell ECM deposition during tissue repair, with control over cell populations and ECM density and composition.

### 0.5 | Lineage Tracing Of Vasculogenic Fibroblasts In Vivo And Their Significance In The Rescue Of Diabetic Ischemic Tissue

Kanhaiya Singh, Sedat Kacar, Sumit S. Verma, Manishkesh Kumar, Sashwati Roy, Chandan K. Sen

1McGowan Institute for Regenerative Medicine, Department of Surgery, University of Pittsburgh, Pittsburgh, PA; 2Indiana University, Indianapolis, IN

Our recent work reported on the identification of the vasculogenic fibroblast (VF) that is capable of generating new blood vessels during tissue repair (Pal et al. Nat Com, 2023). While these cells are physiologic and injury-inducible, the generation of VF is blunted under conditions of diabetes. Such barrier may be overcome by the inhibition of
purposes

Wound Alkalinity Measurement To Prognosticate The Healing Activities of DFUs

Jon Senkowsky2, Shuxin Li1, Wenjing Hu1, Liping Tang1
1Progenitec, Arlington, TX; 2Texas Health Physician’s group, Arlington, TX

PURPOSE: The aim of this study was to determine whether and when wound alkalinity can be measured to prognosticate nonhealing DFUs and to predict healing outcome. METHODS: Wound alkalinity was monitored during the routine visits by assessing discarded wound dressings, via a disposable device - DETEC® pH, from 60 DFU patients to prognosticate healing outcome (probability of complete healing in 12 weeks). RESULTS: To determine the optimal time frame, the test sensitivity, specificity, pre- and post-test healing outcome of these patients were correlated with their wound alkalinity measurements from their 1st, 2nd and 3rd visits. Interestingly, we found that alkalinity assessment from the 2nd visit (7-21 days following the first visit) had the highest sensitivity (77.5%) and specificity (80.0%) to predict a non-healing outcome. The pre-test and post-test risk of a wound not healing was 88.9% and 96.9%, respectively, representing an 8.0% reduced risk of not healing. The improved predictability of the 2nd visit over the first visit can be explained by the wound healing processes. Specifically, following initial wound treatment, such as topical debride ment, it takes at least 7 days for the wound to reach its homeostasis. Those patients with alkaline wounds during their 2nd visit suggest that the wounds did not initiate inflammatory responses after the standard of care and the wounds may have a higher chance of non-healing than those with non-alkaline wounds. Further analysis was carried out to determine the optimal time frame of the 2nd visit for prognostic healing outcome by dividing all DFUs patients (48 patients) based on their visit time frame following the first visit (<7 days: 24 patients; 7-21 days: 24 patients). Our analyses show that, as compared to the <7 days group, the alkalinity determination for the 7-21 days group had highest sensitivity (87.5% vs 72.2%) and specificity (62.5% vs. 50.0%) to prognosticate the wound healing outcome. For 7-21 days group, while the pre-test and post-test risk of not healing was 66.7% and 82.4%, respectively, which represented a 15.7% increased risk of not healing. The risk of not healing when the wounds were non-alkaline was 28.6% which represents a 38.1% reduced risk of not healing. The results support that the 2nd visit (7-21 days after the initial diagnosis/treatment) would be the best time for alkalinity determination with the best non-healing prognostic capability. Using a logistic regression model, we determined that, with all p-values > 0.4, age, gender, race, initial wound size, wound location, and infection status were found to have negligible influence on predicted healing outcome based on wound alkalinity. Conclusions: Our analyses show that alkalinity status can prognosticate healing outcome (p = 0.0436).

0.7 | Anthocyanins From Black Soybean Seed Coat Prevent Radiation-Induced Skin Fibrosis By Downregulating Tgf-B And Smad3 Expression

Jaehoon Choi
Department of plastic and reconstructive surgery, Keimyung University School of Medicine, Daegu, Daegu, Korea (the Republic of)

Background: The aim of this study was to evaluate the protective effects of anthocyanins from the black soybean seed coat against radiation injury in dermal fibroblasts and mouse skin.

Methods: Dermal fibroblasts treated with 50 and 100 μg/mL anthocyanins were irradiated with single doses of 20 Gv. Cell viability, intracellular reactive oxygen species (ROS) production, and mRNA expression were measured. A total of 60 mice were used for an in vivo study. A dose of 100 μg/mL anthocyanins was administered daily for 5 days before or after radiation therapy. Following irradiation (45 Gy), mice were inspected for gross pathology twice per wk for 8 weeks. At 4 and 8 weeks post-irradiation, dorsal skin was harvested for histopathologic examination and protein isolation.

Results: In dermal fibroblasts, treatment with 50 and 100 μg/mL anthocyanins significantly reduced radiation-induced apoptosis at 72 h and intracellular reactive oxygen species generation at 48 h. Furthermore, 100 μg/mL anthocyanins markedly decreased Smad3 mRNA expression and increased Smad7 mRNA expression at 72 h post-irradiation. In mice, treatment with 100 μg/mL anthocyanins resulted in a significant reduction in the level of skin injury, epidermal thickness, and collagen deposition after irradiation. Treatment with 100 μg/mL anthocyanins significantly decreased the number of α-SMA-, TGF-β-, and Smad3-positive cells after irradiation.

Conclusion: Our study demonstrated that black soybean anthocyanins inhibited radiation-induced fibrosis by downregulating TGF-β and Smad3 expression. Therefore, anthocyanins may be a safe and effective candidate for the prevention of radiation-induced skin fibrosis.
ABSTRACTS

0.8 | Granzyme B Mediates Degradation Of Hemidesmosome Proteins In Stevens-Johnson Syndrome/Toxic Epidermal Necrolysis

Michael M. Lane1, Faith Liu1, Alexandre Aubert1, Valerio Russo1, Karen Jung1, Touraj Khosravi2, Hongyan Zhao3, Layla Nabai1, Richard Crawford2, Elizabeth Phillips4, David Granville1
1 Pathology and Laboratory Medicine, The University of British Columbia, Port Coquitlam, BC, Canada; 2 Dermatology and Skin Science, The University of British Columbia, Vancouver, BC, Canada; 3 Department of Pathology, Microbiology and Immunology, Vanderbilt University, Nashville, TN

Background: Stevens-Johnson syndrome (SJS) /Toxic epidermal necrolysis (TEN) are life-threatening, immune-mediated, cutaneous adverse drug reactions characterized by the separation of the epidermal and dermal layers of the skin, resulting in severe blistering and peeling. Serine protease Granzyme B (GzmB) was recently found to contribute to the sub-epidermal blistering in bullous pemphigoid through the cleavage of α6/β4 integrin, collagen VII, and collagen XVII at the dermal-epidermal junction. In the present study, the role of GzmB in SJS/TEN was investigated.

Hypothesis: GzmB accumulation at the dermal-epidermal junction contributes to sub-epidermal blistering in SJS/TEN through the cleavage of α6/β4 integrin, collagen VII, and/or collagen XVII.

Methods: Skin biopsies collected from SJS/TEN patients (n = 8) and healthy participants (n = 8) were analyzed using immunohistochemistry to assess protein levels of GzmB and its substrates, α6/β4 integrin, collagen VII, and collagen XVII. ELISA was used to quantify GzmB levels in blister fluid from patients with SJS/TEN (n = 6) and was compared that of bullous pemphigoid patients (n = 2). Western blotting was used to identify fragments of Collagen XVII in SJS/TEN blister fluid samples (n = 6).

Results: Epidermal and dermal GzmB levels were significantly elevated in SJS/TEN compared to healthy skin. SJS/TEN sections exhibited reduced α6/β4 integrin, collagen VII, and collagen XVII at the dermal-epidermal junction compared to healthy skin. Increased levels of GzmB as well as fragments of collagen XVII (around 120 kDa and 97 kDa, as previously observed in GzmB cleavage assays in vitro) were detected in all SJS/TEN blister fluid samples, suggesting that the extracellular proteolytic activity of GzmB is sustained in SJS/TEN.

Conclusions: The present study provides a novel pathological mechanism of action in SJS/TEN whereby elevated levels of extracellular GzmB mediates degradation of dermal-epidermal junction proteins, leading to separation of the epidermis from the dermis.

P1.01 | SLI-F06, A Fibromodulin-Based Therapeutic Peptide, Enhances Wound Healing In Diabetic Rodent And Pig Models

Pin Ha2, Zhaohan Zeng3, Chenshuang Li3, Joshua Yang2, Evan Yen4, Eric Yen5, Sang Yub Kim2, Elisabeth Leeflang3, Andrew Vardarian2, Kang Ting2, Chia Soo2, ZHONG ZHENG1
2 Scarless Laboratories Inc., Cherry Hill, NJ; 3 University of California, Los Angeles, Los Angeles, CA; 4 University of California, Los Angeles, CA; 5 American Dental Association Forsyth Institute, Cambridge, MA

SLI-F06 is a fibromodulin-derived peptide that effectively promoted wound healing and reduced scar size without discernable adverse effects in multiple preclinical and clinical acute wound studies. It functions through, in part, enhancing fibroblast migration, myofibroblast differentiation, and contraction. Since chronic wounds are deficient in cell migration and wound contraction, we sought to test SLI-F06’s ability to heal cutaneous wounds in challenging diabetic models. Because over 90% of adult diabetic patients in the US exhibit type 2 diabetes mellitus (DM) rather than type 1, we used a NONcNZO10/LtJ type 2 DM mouse model for our first set of wound studies. Two excisional, full-thickness wounds (6 mm diameter) were created on the back of each mouse. To simulate human-type repair in the loose skin mouse, we sewed silicone rings to each wound periphery to “split” the wound to minimize excessive wound margin contraction. Then, we injected SLI-F06 (25 mg/ml) intradermally at four points around the wound edge (25 ml/point; 100 ml total) every other day for 14 days. We documented wound area healing by digital photography and fitted the data to a quantile mixed-effect model with a 95% confidence interval. These data demonstrated that the SLI-F06 application significantly accelerated the wound healing of diabetic NONcNZO10/LtJ mice (P < 0.0001). Moreover, multiple comparisons applying Šidák correction revealed that SLI-F06 markedly enhanced wound healing from Day 1 to 13 (adj. P < 0.05, N = 12). Particularly, during Days 2-8, the quantile mixed-effect model revealed an overall 65.6% (> 30%) faster median wound healing rate with SLI-F06 treatment. Furthermore, the proportion of wounds with complete closure at day 14 post-injury was increased considerably (Mantel-Cox test P = 0.0156; N = 12) at 83.3% in the SLI-F06 group vs. 41.7% in control. Next, we used a streptozotocin-induced diabetic Yorkshire pig model that simulates certain characteristics of later-stage human type 2 non-insulin dependent DM, such as increased triglycerides and glucose intolerance. We excised 1.5 x 1.5-cm square wounds and topical applied 110 ml/cm² of 25 mg/ml SLI-F06 in a hydroxypropyl cellulose excipient twice/week. Excitingly, topical SLI-F06 administration not only accelerated wound healing as assessed by the healed wound area, but also increased the proportion of wounds with complete closure (SLI-F06: 15.0 day vs. control: 18.0 day; Mantel-Cox test P = 0.0300, N = 7). These promising preclinical data strongly support the effectiveness of SLI-F06 in diabetic wound management, which can significantly improve the quality of life of diabetic patients suffering from chronic wounds that can lead to amputations and death.

P1.02 | Linking Human Genetics And Wound Infection With Transcriptome-Wide Associations

Khalid Omeir1, Rebecca Gabrilska2, Jacob Ancira3, Ashley Noe1, Clint Miller2, Craig Tipton1, Kendra Rumbaugh2, Joseph Wolcott3, Nicole Phillips4, Caleb Phillips1
1 Scarless Laboratories Inc., Cherry Hill, NJ; 2 University of California, Los Angeles, Los Angeles, CA; 3 University of Pennsylvania, Philadelphia, PA; 4 Arcadia High School, Arcadia, CA; 5 American Dental Association Forsyth Institute, Cambridge, MA

WHS SESSION P: Concurrent Oral Abstracts III
P1: Chronic Wounds 2
Introduction: Microbes are believed to be key contributors to wound chronicity, yet our understanding about the reasons for inter-patient variation in the species infecting chronic wounds remains unclear. Previous investigations reveal that human genetic variation may partially explain differences in infection and may have to do with how our genetic variation encodes variation in wound bed cellular phenotypes. Because the wound bed is comprised of multiple cell and tissue types, we conducted a microbiome transcriptome-wide association study to investigate how patient genetic variation determines tissue-specific expression and in turn relates to infection. Significant and repeatable gene expression associations may provide insights into the mechanistic details of chronic wound bacterial infection.

Methods: In a deidentified fashion, consenting patients provided buccal swabs and wound debridement samples. The buccal swabs were genotyped at \( \sim 660k \) loci, and chronic wound bacterial microbiota were characterized via 16S sequencing. Whole genome imputation was performed to increase genomic coverage and improve subsequent gene expression imputation. Single nucleotide polymorphisms (SNPs) were used to predict patients’ gene expression levels and splicing patterns for artery, blood, fibroblast, skeletal muscle, skin, subcutaneous fat, and nerve tissue. A balanced and race-stratified two-cohort design of 458 patients was used. Across tissues, there was an average of 12,217 expressed genes and 30,100 splice variants considered. The effect of these genes on transformed relative abundances of 68 bacterial species was evaluated.

Results and Conclusion: The genetically regulated expression of more than 200 differentially expressed genes and splice variants were found to be significantly associated with bacterial relative abundances and reproducible across both cohorts (Bonferroni corrected \( p<0.05 \)). Fifty-six species had significantly associated genes (median number of significant associations per bacterium = 4.5; first quartile = 1; third quartile = 10). Notably, significant genes had roles in cell adhesion and attachment, cell migration, cytokinesis, cytoskeletal integrity, and cytoskeletal dynamics. Findings inform how individual genetic variation translates to differences in bacterial colonization of chronic wounds. Future work will focus on predictive value of SNPs and gene expression for specific infection types.

Background: Diabetic foot ulcers are a common complication of diabetes that is associated with both high morbidity and mortality. It is estimated that the United States alone spends up to \( \$13 \) billion USD each year on DFUs, with individual costs rising to \( \$15,000 \) per year. Much of this expenditure has been attributed to lengthy hospital stays and inpatient care, with the need prolonged antibiotic therapy. Outpatient antibiotic therapy (OPAT) is a service that provides intravenous antimicrobial medication in the outpatient setting and has been shown to reduce hospital stay and overall medical costs.

This study aims to determine the benefit of OPAT therapy in a cohort with soft tissue and bone foot infections.

Methods: Retrospective cohort study of consecutive patients hospitalized for foot infection. We collected demographic data, co-morbidities and one-year outcomes including healing, surgical interventions, number of surgeries, length of stay, re-infection, and re-hospitalization. Patients were grouped by discharge with OPAT program or standard of care. All patients were followed for a minimum of one year. Statistics were performed using Stata Be7. Continuous variables were reported as mean ± standard deviation and categorical variables were reported as their n. Odds ratios were written as O.R, followed by their associated confidence interval [CI]. Significance was determined as \( ps0.05 \).

Results: A total of 382 patients with hospitalized lower extremity infections were analyzed in this cohort. There were 202 subjects with osteomyelitis and 180 subjects with soft tissue infections. OPAT was used in 99 patients. The primary outcomes examined in this study included reinfection, amputation, mortality, rehospitalization of the same foot, and length of stay. The overall events included 168 reinfections, 206 amputations, 9 deaths, 165 rehospitalizations for the target limb. OPAT was significantly associated with reinfection OR = 2.2 [CI: 1.3 - 3.5] and rehospitalization of the same foot O.R = 2.4 [CI: 1.5-3.9].

A sub analysis was further conducted for only the patients with diabetes \( (n=294) \). An association was seen between OPAT and reinfection O.R = 2.1 [CI:1.3-3.6] and rehospitalization for the target limb O. R = 2.4 [CI: 1.4- 4.0] as well. Sub analysis was also performed for patients with osteomyelitis \( (n=202) \). OPAT was protective of index amputation O.R = 0.3 [CI: 0.1-0.6] and associated with rehospitalization of index limb O.R = 2.1 [CI: 1.1- 3.7].

Conclusion: OPAT is associated with reinfection and rehospitalization of the target limb in this cohort. These findings were strongly related to patients with diabetes while no significant findings were observed in patients without diabetes.
diagnosis is often challenging as the clinical presentation can mimic other ulcerative skin disorders with no specific biological marker. Misdiagnosis and delayed diagnosis can induce mismanagement. However, the impact of the delay of PG diagnosis on healing outcomes and subsequent healthcare utilization has not yet been studied and the purpose of our study was to determine this impact.

We conducted a prospective study supplemented by retrospective data for 100 patients with ulcerative PG from the Oregon Health and Science University Pyoderma Gangrenosum Study patient registry. Prospective data included healing time and hospitalizations while ulcer onset, date of diagnosis, and emergency room (ER) visits were collected retrospectively. Total healing time was defined from the date of diagnosis to wound closure. The patients were divided between a delay of diagnosis ≤3 months and >3 months based on the definition of a chronic wound. Healing time was evaluated with a Kaplan-Meier analysis.

Twenty-six percent of patients had a diagnostic delay of ≤3 months. The average ulcer size (available for 84% of patients) was 56cm² (95% CI 10 to 103) for ≤3 months and 71cm² (95% CI 50 to 92) for >3 months diagnostic delay. The average pain in a numeric rating scale was 3.6 and 4.4 out of 10 for each group respectively (p = 0.213). There was a statistically significant difference in median time to healing between the groups: 14.0 months (95% CI 9.4 to 26.6) with a delay of diagnosis ≤3 months and 21.7 months (95% CI 10.2 to 62.9) with a delay of diagnosis >3 months (log-rank P = 0.014). The difference in number of ER visits was also statistically significant with a mean of 0.5 visits for a delay of diagnosis ≤3 months and 1.5 visits for a delay of diagnosis >3 months (P = 0.038). Moreover, the number of hospitalizations was significantly higher for patients with a diagnostic delay >3 months [2.3 hospitalizations versus 1.4 hospitalizations (P = 0.034)].

Our study demonstrated that a delay of PG diagnosis >3 months by medical providers prolongs healing time and significantly increases the number of ER visits and hospitalizations. As a result, delayed diagnosis may lead to higher costs and an increased burden on the healthcare system influencing treatment time and treatment costs for patients. Given average pain is similar in both groups, patients with a longer disease course as a result of delayed diagnosis have prolonged suffering. Delayed healing time could also lead to increased time lost from work. In conclusion, this study clearly shows the negative consequences of a delayed PG diagnosis supporting why it is paramount to increase awareness of this underdiagnosed disease among medical providers and the necessity of specific markers to aid in a timely diagnosis.

P1.05  |  LCM-Directed Profiling Of Acute And Chronic Wounds Identifies Proteomic And Lipidomic Signatures Of Healing And Non-Healing Wounds

Veronika Jurczuk1, Lilian Valadar Tose2, Leticia L. Rodriguez3, Beatriz Abdo Abujamra1, Maria Bouлина3, Sinan K. Jabori4, Devinder Singh4, Sara Danker4, Francisco Fernandez Lima2, Ivan Jozic1

1Dermatology, University of Miami School of Medicine, Miami, FL; 2Chemistry and Biochemistry, Florida International University, Miami, FL; 3Diabetes Research Institute, University of Miami, Miami, FL; 4Division of Plastic and Reconstructive Surgery, University of Miami, Miami, FL

Purpose: The purpose of our study was to utilize unbiased proteomic and lipidomic approaches to identify potential biomarkers of healing and non-healing wounds.

Methods: For acute wounds, we topically treated ex vivo human skin with deuterium oxide (D2O), whose incorporation into newly synthesized macromolecules was assessed at 24, 48, 72 and 96h post treatment (n=4). Similarly, for chronic wounds we utilized diabetic foot ulcer (DFU) and venous leg ulcer (VLU) specimens (n=3). Harvested tissues were cryosectioned & freeze dried, with areas of interest dissected using LCM microscopy and sequential sections subjected to either lipidomic or proteomic analysis using time of flight secondary ion mass spectrometry (ToF-SIMS) or trapped ion mobility spectrometry (timsToF-Ultra) with a high-spatial resolution LMIG analytical beam (respectfully). Mass-to-charge ratio (m/z) was utilized to identify various lipid and peptide species using Spectronaut 18, thus allowing for hierarchical clustering of samples, followed by STRING-DB and DAVID analysis for functional annotation of identified clusters of different lipidomic and proteomic species.

Results: TOF-SIMS detected fatty acids 16:0 and 16:1 (255.2, 253.2 m/z), 18:0, 18:1 and 18:2 (283.3, 281.2, 279.2 m/z) and cholesterol sulfate (465.3 m/z) in both acute and chronic wounds with the latter exhibiting close to 200-fold increase in epidermis of both DFUs and VLUs. Conversely, long chain fatty acids (24:0, 25:0, 26:0 and 28:0), as well as ceramides were almost devoid from the healing epidermis and were primarily localized to granular and cornified layers away from the healing epidermis in acute wounds and throughout the entirety of both DFUs and VLUs. On the other hand, proteomic analyses identified a number of proteins that exhibited differential expression in either healing epidermis or dermis in comparison to their counterparts away from the wound (including but not limited to: ALDOC, ANXA3, ATPA, BAF, CALD1, CALM1, CAV1, DESP, EPIPL, FAK2, G3PT, GELS, H11, K1C24, KRT36, K2C6A, PDIA1, POSTN, RL12, ROA1, SPB5, VINC) with a relative difference in abundance >2.0 fold. Moreover, we were able to identify a number of differentially expressed peptides between acute and chronic wounds, within both epidermal and dermal compartments (A1AG, CALM2, CCN1, DCD, FIBB, FNDC1, H2A1J, HBB, K1C9, K22E, MACF1, OR2T2, O2T35, PGS2, RL2A, S10A9, VIME, among others).

Conclusions: Together our data identify a novel method for detection of proteomic and lipidomic changes in acute and chronic wounds. Moreover, identified signatures serve as targets for development of novel topical formulations for accelerated wound closure, which we are actively pursuing.

P1.06  |  Staphylococcus Epidermidis Fitness In The Chronic Wound Microenvironment Is Driven By Antimicrobial Resistance Traits

Jamie L. Burgess2, Miroslav Dinić1, Rebecca Verpile2, Tammy Gonzalez2, Jingjing Ming2, Jelena Marjanovic2, Carmen Beliz2,
A perturbed microbiome with persistent wound infection by biofilm forming bacteria is characteristic of chronic wounds, including venous leg ulcers (VLUs). *Staphylococcus epidermidis* is the most abundant skin-resident bacteria primarily known to be beneficial to the host but is also recognized as an “accidental” pathogen in certain environments. As the role of *S. epidermidis* in the wound chronicity is still unclear, we performed an in-depth characterization of *S. epidermidis* isolated from chronic VLUs. We utilized a human ex vivo wound model to show that healthy, commensal strains of *S. epidermidis* were incapable of surviving the human ex vivo wound environment. However, *S. epidermidis* strains isolated from VLUs showed a biofilm-dependent induction of IL-1β, IL-8 and IL-6 and inhibition of wound re-epithelialization, correlating with the healing outcome of the corresponding VLU patients. Using whole genome sequencing, we found both commensal and VLU isolates of *S. epidermidis* had similar signatures for biofilm formation and adhesion, but only the VLU isolates demonstrated higher biofilm formation and extracellular matrix binding. A majority of the VLU patients tested (n=24) had a high prevalence of mupirocin and mexitilin resistance genes, contributing to the emergence of treatment-resistant virulent lineages in patients with non-healing ulcers. Our data emphasizes the need to develop therapeutics targeting bacterial attachment or mechanical removal by debridement rather than a bactericidal approach to infection in VLUs.

P2: Fibrosis/Scarring 2

P2.01 | Characterizing Vascular Leakage After Angiopoietin-1 Silma Knockdown In Dermal Microvascular Endothelial Cells Derived From Post-Burn Hypertrophic Scar

Esteban Molina1, Lauren Moffatt2, Jeffrey W. Shupp3, Bonnie Carney2
1Georgetown University School of Medicine, Clifton, VA; 2Firefighters’ Burn and Surgical Research Laboratory, MedStar Health Research Institute, Washington; 3The Burn Center, Department of Surgery, MedStar Washington Hospital Center, Washington DC, United States

Recent data demonstrate that dermal microvascular endothelial cells (DMVECs) from hypertrophic scars (HTSs) have increased angiopoietin-1 (ANGPT-1) gene and protein expression compared to normal skin (NS) DMVECs and lower permeability of FIT-C dextran using a transwell assay. This study evaluated the effect of ANGPT-1 knockdown on endothelial cell permeability to characterize the role of endothelial dysfunction in HTS. Full-thickness burns which formed HTSs were created in Duroc pigs. Punch biopsies were taken at day 84 post-burn and stained with Verhoff Van Geison stain (VVG). HTS and areas of NS were excised and digested in collagenase. Fibroblast-DMVEC co-cultures were saved in cryostorage. Ulex europeaus agglutinin 1 lectin was used to sort for DMVECs by magnetic-activated cell sorting. For the knockdown, DMVECs were grown in 6-well plates with n=3 wells per experimental (treatment, control, scramble) group. Treatment group was treated with siRNA for ANGPT-1. At 24 hours post-transfection, RNA was isolated. Gene expression of ANGPT-1 was quantified using qRT-PCR. In the permeability assay, DMVECs from HTS were seeded onto 12-well transwell plates. Trans-endothelial electrical resistance (TEER) was assessed on day 1 to assess the formation of a monolayer. On day 3 post-seeding, transwells were treated with either siRNA for ANGPT-1 (n=8), H2O as control (n=7), or scramble siRNA (n=7). On day 4, 24 hours post-transfection, FIT-C was added to the apical well and dwelled for 2 hours. FITC-dextran that diffused into the bottom well was measured by spectrophotometry. Student's t-test was used to evaluate gene expression between knockdown vs control group with p<0.05 considered significant. Transwell concentrations between experimental groups were analyzed using one-way ANOVA with Tukey's correction for multiple comparisons. Punch biopsies from scars showed significantly increased blood vessel density compared to normal skin on VVG stain (p<0.001). DMVEC identity was confirmed by immunofluorescence staining for Von-Willebrand factor. ANGPT-1 gene expression showed downregulation >4-fold for siRNA knockdown group compared to control group (p=0.21) while scramble did not show ANGPT-1 knockdown. TEER on day 1 averaged 17.28±4.5 Ω/cm² for n=12 wells and 28.97±5.3 Ω/cm² for n=12 wells. siRNA knockdown group showed increased permeability to FIT-C dextran compared to scramble siRNA (-23.39±3.9 vs 4.24±1.6; p=0.0002). Knockdown using siRNA for ANGPT-1 in HTS DMVECs led to an increase in endothelial permeability compared to the scramble group. Additional siRNA targets outside of the angiopoietin group will be tested in future work to identify candidate drug targets for treating endothelial dysfunction in scars.

P2.02 | Endothelial Cell-Derived Extracellular Vesicles And Their Micromas Downregulate Fibrotic Pathways In Fibroblasts

Heidi Yuan, Anna Salapatas, Trevor R. Leonardo, Chen Han, Devina Koshal, Matusz S. Wietecha, Lin Chen, Siram Ravindran, Luisa A. DiPietro
University of Illinois Chicago, Chicago, IL

Background: An adequate wound healing response requires the coordination of intercellular signals between multiple cell types. During the proliferative and remodeling phases of healing, endothelial cells and fibroblasts are likely to communicate. We hypothesize that this communication occurs through the release of endothelial cell-derived extracellular vesicles (ECEVs), which contain microRNAs that...
transcriptionally regulate fibrotic gene signatures and influence fibroblast behavior.

**Methods:** ECEVs were collected from primary human dermal microvascular endothelial cells using Exoquick-TC and validated using Nanoparticle Tracking Analysis (NTA), Western blot (WB), and transmission electron microscopy (TEM). Bulk RNA sequencing was performed on human dermal fibroblasts treated with ECEVs. ECEVs were also sequenced to determine microRNA content. The relative expression of select gene targets and ECEV microRNAs was validated with qRT-PCR of fibroblasts treated with ECEVs for 24 hours. Fibroblast function was assessed following transfection of a microRNA candidate that had increased expression in ECEV-treated fibroblasts.

**Results:** Ingenuity Pathway Analysis (IPA) was used to identify top Canonical Pathways affected in fibroblasts treated with ECEVs. The microRNA target filter in IPA was then applied to determine the pathway-specific transcriptional targets of the top 20 microRNAs found in ECEVs. The analysis identified Pulmonary Fibrosis Idiopathic, Hepatic Fibrosis, and Wound Healing as the top 3 downregulated pathways in ECEV-treated fibroblasts, suggesting that ECEVs exert anti-fibrotic effects on fibroblasts. Among these three pathways, 11 common ECEV microRNAs were predicted to target genes related to fibroblast proliferation, differentiation into myofibroblasts, contraction, and formation of collagen. Five genes (ACTA2, MAP2K6, PDGFA, TGFBR3, COL1A2) demonstrated reduced relative expression in ECEV-treated fibroblasts when validated using qRT-PCR (p<0.05). MiR-126-3p was the most highly expressed microRNA in ECEVs, comprising over 20% of total microRNA content. Intracellular levels of miR-126-3p in fibroblasts were also elevated (p<0.05) following ECEV treatment, and transfection of fibroblasts with mimics of miR-126-3p resulted in impaired migration and proliferation (p<0.05).

**Conclusions:** ECEVs contain microRNAs that are predicted to repress pro-fibrotic gene signatures in fibroblasts. Preliminary *in vitro* validation suggests that the transfer of miR-126-3p from ECEVs to fibroblasts is one mediator of this anti-fibrotic effect. In the context of wound healing, this endothelial cell-fibroblast communication may be a means of precluding early ECM deposition or contraction, allowing proper vascular remodeling in the wound bed.

**P2.04 | Cancer and Lymphatic Marker FOXC2 Drives Wound Healing And Fibrotic Tissue Formation**

Maia Granoski¹, William Hahn¹, Katharina Fischer¹, Hudson Kussie², Dharshan Sivaraj³, Andrew Hostler¹, Eamonn McKenna³, Jonathan P. Yasme⁴, Robert Erickson¹, Marlys Witte¹, Geoffrey C. Gurtner³, Kellen Chen²

¹Surgery, University of Arizona, Tucson, AZ; ²Stanford University, Palo Alto, CA

**Background:** Wound repair is a complex process that engages many different physiological systems as the tissue progresses through a series of interdependent phases, including inflammation and remodeling. The FOXC2 transcription factor has been tied to tissue development during embryogenesis, and has been clinically associated with aggressive basal-like human breast cancers. Systemic dysregulation of FOXC2 expression has also been found to promote defects in lymphatic remodeling and hyperplastic lymphedema-distichiasis (LD). Since chronic lymphedema is a forerunner of several malignancies and cancers have been known to arise from poorly healing chronic wounds, we examined the effect of Foxc2 dysfunction on skin wound healing.

**Methods:** We used our splinted excisional wounding model that mimics human-like wound healing on wildtype and Foxc2 +/- mice, which demonstrate incomplete lymphatic vasculature, lymphatic dysfunction, and enhanced cancer metastasis. Wound size was measured over the course of 18 days. Tissue was explanted from both groups at post-operative day (POD) 14 and 18 and stained with Masson’s Trichrome to assess scar formation, Picrosirius Red for dermal integrity, or immunofluorescence to assess macrophage (F4/80) cell populations.
**Results:** Wildtype mice had completely healed wounds by POD 14, while Foxc2+/− mice did not heal until POD 18 (p=0.0104). On PODs 8 (p=0.0001), 10 (p=0.0001), 12 (p=0.0001), and 14 (p=0.0002), the wound size of Foxc2+/− mice was significantly larger than that of wildtype mice. Scar area of healed Foxc2+/− mice (POD 18) was significantly larger than that of healing Foxc2+/− mice (POD14; p=0.0098) and that of healed wildtype mice (POD 14; p=0.0294). Collagen fibers in the healing scar of Foxc2+/− mice were narrower (p=0.0117) and more highly aligned (p=0.0110), indicating significantly more fibrosis in Foxc2+/− mice compared to wildtype mice. Collagen fibers in both groups became significantly longer (p=0.0116) and wider (p=0.0020) over time, indicating a temporal evolution of fibrosis during the remodeling phase of wound healing. Foxc2+/− mice also had significantly lower numbers of F4/80 cells (p=0.0014) compared to wildtype mice, indicating poor immune cell infiltration at the wound site.

**Conclusion:** We found that FOXC2, which is tied to cancer metastasis and lymphatic dysregulation, also impairs wound healing and promotes fibrotic tissue architecture, linking these disease states together. With FOXC2 proposed as a potential therapeutic target for cancer metastasis, its pleiomorphic downstream systemic effects should be considered and weighed against the increased risk of developing nonhealing wounds, potentially by inhibiting the immune system. Further delineation of the microenvironment, cellular events, and molecular signals during normal and Foxc2-associated abnormal wound healing will improve clinical therapies targeting this marker.

**P2.05 | Inhibition Of CYP24A1, An Enzyme Involved In Vitamin D Metabolism, Alters Profibrotic Gene Expression In Keloid-Derived Keratinocytes**

Dorothy Supp¹, Jennifer Hahn¹, Kelly A. Combs², Heather Powell²

¹Surgery, University of Cincinnati College of Medicine, Cincinnati, OH; ²Materials Science Engineering and Biomedical Engineering, The Ohio State University, Columbus, OH

**Introduction:** Keloids are disfiguring fibroproliferative lesions that can occur following injury to skin in susceptible individuals. Keloids are challenging to treat and recurrence after treatment is common. A deeper understanding of the molecular mechanisms driving keloid formation is necessary for development of more effective therapies for keloid suppression. We previously identified reduced expression and decreased nuclear localization of the vitamin D receptor (VDR) in keloid epidermis, implicating vitamin D signaling in keloid pathology. Here we report that CYP24A1 is overexpressed in keloid keratinocytes compared with normal keratinocytes. The CYP24A1 gene encodes 24 hydroxylase, a vitamin D metabolizing enzyme that degrades 1,25-dihydroxyvitamin D3 (1,25-D3), the active form of vitamin D. The CYP24A1 gene is itself induced by vitamin D in a feedback loop that regulates 1,25-D3 levels. In this study, we investigated the effects of CYP24A1 inhibition in normal and keloid-derived keratinocytes.

**Methods:** Normal and keloid keratinocytes (N=3 donors each) were cultured +/− 1.25-D3 and +/− inhibitors of CYP24A1, which included ketoconazole, a non-specific inhibitor of cytochrome P-450 enzymes, and VID-400, a specific inhibitor of CYP24A1. Proliferation was measured using an MTT assay, and gene expression was analyzed by quantitative PCR. Statistical analyses were performed using t test (2 groups) or One Way ANOVA (>2 groups) using SigmaPlot 15.0.

**Results:** CYP24A1 mRNA was expressed at 3.5X higher levels in keloid keratinocytes compared with normal keratinocytes. Ketoconazole inhibited proliferation of keloid and normal keratinocytes, but VID-400 had no significant effect on keratinocyte proliferation. The two inhibitors had different effects on expression of vitamin D target genes in keratinocytes. For example, ketoconazole treatment alone reduced expression of CYP24A1 in normal and keloid keratinocytes, whereas VID-400 treatment increased CYP24A1 expression. Both inhibitors decreased expression of the profibrotic genes Periostin (POSTN) and Hyaluronan synthase 2 (HAS2), previously shown to be upregulated in keloid-derived cells. Combined treatment of keloid-derived keratinocytes with 1.25-D3 and ketoconazole or VID-400 increased the effects of 1.25-D3 treatment on vitamin D target genes and profibrotic gene expression, although the effects were gene- and cell type-specific.

**Conclusions:** The data suggest that reduction of 1.25-D3 inactivation with inhibitors of CYP24A1 may reduce profibrotic gene expression in keloid-derived cells. Because vitamin D has numerous anti-inflammatory and antifibrotic activities, inhibitors of CYP24A1 may serve as adjunctive therapies to suppress keloid-associated gene expression changes.

**P2.06 | Extracorporeal Shock Wave Therapy Alleviate Radiation-Induced Skin Fibrosis By Downregulating TGF-B Expression**

Sangwoo Park

Department of Plastic and Reconstructive Surgery, Samsung Changwon Hospital, Sungkyunkwan University School of Medicine, Changwonsi, Changwonsi, Korea (the Republic of)

**Background:** The aim of this study was to evaluate the protective effects of extracorporeal shock wave therapy (ESWT) against radiation injury in dermal fibroblasts and mouse skin.

**Methods:** Dermal fibroblasts were treated with ESWT (1000 impulses at 4Hz and 0.1mJ/mm²) after irradiation (20 Gy). Cell viability, cell migration, and mRNA and protein expression were measured. A total of 24 mice were used for an in vivo study. Mice were treated with ESWT (200 impulses at 4Hz and 0.25mJ/mm²) daily for 2 weeks after irradiation (45 Gy). At 8 weeks post-irradiation, dorsal skin was harvested for histopathologic examination and protein isolation.

**Results:** In dermal fibroblasts, the viability of irradiated cells was significantly increased after treatment with ESWT, relative to irradiated, nontreated cells (p=0.005). ESWT significantly reduced TGF-β1 protein expression at 48h post-irradiation (p=0.024). ESWT significantly
increased cell migration at 24h post-irradiation (P = 0.002). In mice, the irradiated skin treated with ESWT exhibited decreased collagen deposition compared with the control. ESWT significantly reduced TGF-β1 (p = 0.001) and phospho-Smad3 (p = 0.004) protein expression after irradiation. ESWT significantly decreased the number of TGF-β1- and α-SMA-positive cells after irradiation (p < 0.001).

Conclusions: Our study demonstrated that ESWT inhibited radiation-induced fibrosis by downregulating TGF-β1 expression. Therefore, ESWT may be a safe and effective candidate for the prevention of radiation-induced skin fibrosis.

P3: Inflammation

P3.01  |  Innate Immune Interactions Define Key Mechanisms Of Exosome-Induced Healing Of Chronic Wounds

Kody P. Mansfield, Dianny Almanzar, Bibi S. Subhan, Jasmina Abdalla, Juan Troncoso, Lesly Honore, Piiul S. Rabbani
Hansjörg Wyss Department of Plastic Surgery, NYU Langone, Brooklyn, NY

Chronic wounds, thus far, are managed clinically but are not treated for underlying pathology. Specifically, in the population with diabetes, chronic wounds do not manifest the repair or regenerative properties of skin and thus, are one of the leading causes of non-traumatic lower limb amputations. Major hallmarks of these chronic wounds include neuropathy, peripheral artery defects, and immune dysfunction. We found that local administration of secreted nanoscale vesicles, called exosomes, harvested from cultured primary human bone marrow multipotent stromal cells, accelerate wound closure in a genetic animal model of type 2 diabetic delayed wound healing. The cellular and molecular sequelae of exosome-based treatment, however, remain to be investigated and is at the heart of our study. Cutaneous administration of exosomes at post-operative day 1 (POD1) significantly decreased time to closure and wound burden of type 2 diabetic wounds, in a dose dependent manner compared to PBS-treated control diabetic wounds. In order to characterize exosome-induced healing mechanisms, we employed multiplexed immunofluorescence imaging and single cell RNA sequencing. Our analysis revealed a pro-healing phenotype, likely modulated through interactions between innate immunity and cutaneous vasculature. Immunofluorescence imaging of exosome-treated wounds displayed extensive CD31+ neovascularization in expanded areas of granulation tissue in the diabetic wound bed by POD10, phenocopying typical healing wounds of wild-type mice. We also observed increased presence of undifferentiated monocytes/macrophages and macrophages in the diabetic wound tissue by 1.6-fold and 6.2-fold of that in control wounds, respectively. In vitro studies confirmed that exosomes were capable of modulating primary murine macrophages to a pro-healing-like phenotype. Further confirming our hypothesis of innate immune activation, exosome treatment failed to rescue the delayed healing in clodronate liposome-treated, macrophage-depleted mice. These macrophage-depleted but exosome-treated wounds also lack the development of highly vascularized granulation tissue indicating that macrophage induction is paramount for exosome-induced wound healing. Interestingly, even the endogenous control of macrophage plasticity throughout the sequential phases of skin healing remains elusive even in the context of non-pathological or typical healing wounds. Our results demonstrate that macrophage modulation is at least one avenue through which exosomes mediate wound closure benefit. Our results begin to address the obvious need of studies of cellular events engaged following exosome-induced wound healing, a critical barrier in the safe clinical translation of this tissue engineering approach.

P3.02  |  Topical Fluoxetine Dosage and Administration Chronobiology Affects Cutaneous Wound Healing

Moyasar A. Alhamo1, Anthony Gallegos1, Hsin-ya Yang1, Elham Aslankihi2, Marcella Gomez2, Marco Rolandi2, Rivkah Isseroff2
1UC Davis Health, Sacramento, CA; 2UC, Santa Cruz, CA; 3UC, Santa Cruz, CA

Cutaneous wound healing requires the coordinated processes of cell proliferation and migration. Fluoxetine (FLX), a selective serotonin reuptake inhibitor widely used for treatment of mood disorders, has also been shown to have immunomodulatory properties. Moreover, research from our group has shown that FLX enhances the migration of keratinocytes and promotes healing in murine wounds. Therefore, it is reasonable to propose that applying FLX topicaly during the inflammatory phase of wounds may both modulate wound inflammation and improve re-epithelization. However, the optimal FLX dosage and administration time remain to be elucidated. Our investigation focused on assessing local wound gene expression and re-epithelization, with the hypothesis that dosage and administration timing may modulate key healing parameters. Using a pig wound model, twelve circular wounds measuring 20 mm diameter each were created on the dorsal skin of young female Yorkshire pigs. FLX was applied topically at either high dose (0.45 mg/wound/day) or low dose (0.025 mg/wound/day) during specific time intervals (days 0-2, 3-6, or 7-9). Despite observing a decreased mRNA expression of the macrophage 1(NOS2)/macrophage 2 (ARG-1) ratio in the high-dose FLX groups during days 0-2, 3-6, or 7-9 compared to low-dose groups, we found that the low-dose FLX group exhibited the highest wound re-epithelization at 49.2% of original wound size on days 3-6, surpassing the high-dose groups (on days 0-2, 3-6, or 7-9). Similarly, higher mRNA expression of the early neuronal marker (DXC), essential for neuronal migration, was observed in low-dose groups (on days 0-2, 3-6, or 7-9) compared to high-dose groups. Together, despite a decreased macrophage 1(NOS2)/macrophage 2 (ARG-1) ratio in high-dose FLX groups suggesting down modulation of the inflammatory response under that condition, the low-dose FLX group demonstrated improved wound re-epithelization at 49.2% when the drug was applied during days 3-6 post wounding, outperforming the high-dose groups across various time intervals. Additionally, increased
expression of the early neuronal marker (DXC) in low-dose groups throughout different time points suggests a potential association between lower FLX doses and enhanced neuronal migration into the wound. This line of inquiry provides valuable insights into the impact of FLX dosage and administration chronobiology on the wound healing process.

Our data demonstrate impaired keratinocyte activation and GDT cell infiltration in the absence of P-2 that is more pronounced in aged mice, indicating a novel mechanism relying on the P-2 and Skint-1 in modulating the inflammatory response upon wounding.

Butyrophilin (BTN)-related Skint-1 (Selection and upkeep of intraepithelial T cells 1) molecule is expressed on epidermal keratinocytes and thymic epithelial cells. Skint-1 specifically drives the development of the subtype of gamma delta T (GDT) cells, dendritic epidermal T cells (DETCs) progenitors in the thymus. Importantly, homeostasis and activation of resident epithelial GDT cells are under the control of butyrophilin (BTN) and butyrophilin-like (BTNLI) molecules, in humans and mice. However, it is not well understood how Skint-1 influence DETCs function in the epidermis in response to wounding. Interactions between commensal microbiota and the multiple cell types involved in cutaneous wound healing regulate the immune response and promote barrier restoration. In that regard, we have recently shown that Perforin-2 (P-2), novel anti-microbial protein expressed in the skin, is critical for the clearance of intracellular pathogens. We postulated that P-2 contributes to GDT cell activation and recruitment in response to wounding through regulation of Skint-1.

We induced full thickness wounds on the dorsal skin of 2- or 10-month-old C57/BL6 WT and P-2 knock-out (KO) mice and assessed healing at days 3 and 6 post wounding. We analyzed re-epithelialization and keratinocyte activation using histomorphometry and keratin-6 staining. We expressed Skint-1 on the HEK-293 cells and co-cultured skin GDT cells in the presence of anti-Skint-1 antibody and measured activation of GDT cells by multicolor flow cytometry.

We found that in aged 10-month-old mice, wound re-epithelialization was significantly delayed at day 6 in P-2 KO mice compared to aged WT and young P-2 KO (p<0.05). The healing delay was accompanied by significantly decreased keratin-6 expression at the wound edge (p<0.05). Immunofluorescence staining and flow cytometric analysis revealed significant reduction in GDT cells in the wound bed in aged P-2 KO mice (p<0.05). The GDT cells in the P2 KO background also displayed lower expression of activation markers, including significantly lower mRNA and protein levels of Skint-1 on day 6 post-wounding (p<0.05). In vitro co-culture experiments confirmed that epithelial Skint-1 induces activation of GDT cells.

Butyrophilin (BTN)-related Skint-1 (Selection and upkeep of intraepithelial T cells 1) molecule is expressed on epidermal keratinocytes and thymic epithelial cells. Skint-1 specifically drives the development of the subtype of gamma delta T (GDT) cells, dendritic epidermal T cells (DETCs) progenitors in the thymus. Importantly, homeostasis and activation of resident epithelial GDT cells are under the control of butyrophilin (BTN) and butyrophilin-like (BTNLI) molecules, in humans and mice. However, it is not well understood how Skint-1 influence DETCs function in the epidermis in response to wounding. Interactions between commensal microbiota and the multiple cell types involved in cutaneous wound healing regulate the immune response and promote barrier restoration. In that regard, we have recently shown that Perforin-2 (P-2), novel anti-microbial protein expressed in the skin, is critical for the clearance of intracellular pathogens. We postulated that P-2 contributes to GDT cell activation and recruitment in response to wounding through regulation of Skint-1.

We induced full thickness wounds on the dorsal skin of 2- or 10-month-old C57/BL6 WT and P-2 knock-out (KO) mice and assessed healing at days 3 and 6 post wounding. We analyzed re-epithelialization and keratinocyte activation using histomorphometry and keratin-6 staining. We expressed Skint-1 on the HEK-293 cells and co-cultured skin GDT cells in the presence of anti-Skint-1 antibody and measured activation of GDT cells by multicolor flow cytometry.

We found that in aged 10-month-old mice, wound re-epithelialization was significantly delayed at day 6 in P-2 KO mice compared to aged WT and young P-2 KO (p<0.05). The healing delay was accompanied by significantly decreased keratin-6 expression at the wound edge (p<0.05). Immunofluorescence staining and flow cytometric analysis revealed significant reduction in GDT cells in the wound bed in aged P-2 KO mice (p<0.05). The GDT cells in the P2 KO background also displayed lower expression of activation markers, including significantly lower mRNA and protein levels of Skint-1 on day 6 post-wounding (p<0.05). In vitro co-culture experiments confirmed that epithelial Skint-1 induces activation of GDT cells.
P3.05  |  Excessive Levels Of Neutrophils Cause Macrophage Dysfunction In Fibrotic Volumetric Muscle Loss (VML) Injury

Ricardo Whitaker, Kara L. Spiller  
School of Biomedical Engineering, Sciences and Health Systems, Drexel University, Philadelphia, PA

Volumetric Muscle Loss (VML) is a debilitating condition defined by the rapid loss of muscle mass, leading to permanent impairment. The current standard of care is insufficient due to the poor understanding of the molecular/cellular processes governing VML repair. Macrophages are crucial for muscle repair due to its interaction with neighboring, and changes in macrophage phenotype have been observed following critical size VML injuries. The goal of this study is to uncover mechanisms driving macrophages towards a dysfunctional phenotype following VML.

We employed a murine model of VML using subcritical size injuries (Regenerative), and critical size injuries (Fibrotic). We utilized flow cytometry to evaluate immune cell trafficking and macrophage phenotype in muscle over 28 days, and for 7 days in the spleen and bone marrow. Multiplex gene expression (NanoString) was used to characterize FACS-sorted macrophages and whole muscle tissue over 3 days. Systemic cytokines were quantified via multiplex cytokine analysis (Luminex) over 7 days. Finally, tissue healing was assessed via histology on Day 28.

Fibrotic muscle macrophages demonstrated a higher expression of inflammatory and lower expression of reparative markers over 28 days. Noticeably, similar results were observed in the spleen over 7 days. Hierarchical clustering of muscle macrophages also uncovered hybrid macrophage phenotypes, particularly at Day 7. At the gene level, Fibrotic macrophages in muscle presented a broad downregulation as early as Day 1 compared to Regenerative injury. These results demonstrated that macrophage dysfunction appears soon after injury and prior to onset of fibrosis.

Whole muscle and blood analyses uncovered a higher expression of neutrophil chemokines and systemic G-CSF after Fibrotic injuries, accompanied by an increase in neutrophil influx to the muscle. These results suggest an active role of neutrophils in the progression of this pathology, and perhaps in modulating macrophage phenotype. To evaluate neutrophil-macrophage crosstalk, we partially depleted neutrophils so that neutrophil accumulation in the muscle following Fibrotic injuries resembles levels observed in Regenerative group. Partial neutrophil depletion decreased macrophage expression of inflammatory markers/genes and increased reparative marker/genes expression. However, no improvement in muscle physiology was observed, suggesting that neutrophil depletion improves macrophage phenotype, but is not enough to leverage muscle healing.

Here we have, for the 1st time, observed systemic changes in immune behavior following VML injury. Moreover, we uncovered neutrophils as critical macrophage modulators. These results can instruct the development of better therapeutics.

P3.06  |  Transcriptomic Predictors Of Oral Mucositis Severity In Head And Neck Cancer Patients

Taichi Goto1, Patricia Corby2, Alexander Lin3, John Lukens3, Stephen Sonis4, Leorey N. Saligan1  
1Symptoms Biology Unit, NIH/NINR, Bethesda, MD; 2Department of Radiation Oncology, University of Pittsburgh, School of Medicine, Pittsburgh, PA; 3Department of Radiation Oncology, University of Pennsylvania, Perelman School of Medicine, Philadelphia, PA; 4Brigham and Women’s Hospital and the Dana-Farber Cancer Institute, Boston, MA

BACKGROUND: A common side effect of radiation therapy for head and neck cancer (HNC) is oral mucositis (OM), characterized by erythema, edema, and ulcers. Severe OM affects more than two-thirds of HNC patients, which can lead to malnutrition, dehydration, and risk of infection. Preliminary evidence suggests the role of transcriptome changes in pain heterogeneity associated with OM, but the literature lacks evidence if transcriptomic factors play a role in the development and worsening of OM. The present study aimed to identify differentially expressed genes (DEGs) before cancer treatment (baseline) that may be associated with the worsening of OM during radiation therapy (RT), and 2) to determine the gene sets associated with the identified DEGs.

METHODS: This is a sub-analysis of a clinical trial (ARMOR-Trial NCT03843554), where HNC patients who were expected to receive at least 5000 cGy of RT to the oral or oropharyngeal mucosa were recruited. This study used data from 26 participants in a control group that received regular oral care. Total RNA extracted from whole blood at baseline (before RT) was subjected to RNA-seq analysis. Based on changes in OM severity from baseline to RT completion assessed by the World Health Organization’s Oral Toxicity Scale, the participants were classified into mucositis (change ≥ 1, n = 20) or no mucositis groups (change < 1, n = 6). DEGs were identified between the two groups with a p-value < 0.05 and |log2 fold change| > 1. Gene Set Enrichment Analysis (GSEA) determined gene sets associated with the identified DEGs.

RESULTS: A total of 161 (132 upregulated and 29 downregulated) DEGs were identified. GSEA showed 13 activated biological processes, including “peptide metabolic process (normalized enrichment
score (NES = 1.73, p = .006), “regulation of hormone levels (NES = 1.76, p = .008),” and “homeostatic process (NES = 1.58, p = .045).” GSEA also showed four activated and one suppressed molecular function, including activated “cytokine activity (NES = 1.78, p = .007)” and suppressed “enzyme binding (NES = -1.94, p = .003);” and four activated and one suppressed cellular components, including activated “binding membrane of organelle (NES = 1.74, p = .015)” and suppressed “somatodendritic compartment (NES = -1.71, p = .023).”

CONCLUSIONS: We profiled the transcriptomic signatures before RT that are related to worsening OM severity in HNC patients. The GSEA suggested that some homeostasis-related biological activities, including metabolism, hormone regulation, and cytokine activities were associated with the worsening of OM in this population, which is informative for further investigations.

P4: Burn and Acute Wounds

P4.01 | 5-Lipoxygenase Exerts Sex-Dependent Effects On Burn Wound Healing

Shannon M. Clayton1, Kristina Sanchez2, Maliha Newsome2, Niyab Ahad2, Athena Soulika1
1Dermatology, UC Davis, Sacramento, CA; 2Shriners Hospital for Children Northern California, Sacramento, CA

5-lipoxygenase (5-LO) is the rate-limiting enzyme for the synthesis of bioactive lipid mediators. These mediators are members of the eicosanoids, which are bioactive lipids, derived from polyunsaturated fatty acids that promote and/or regulate inflammation.

Previous research suggests that following in vitro stimulation, 5-LO activity is increased in female neutrophils and monocytes of humans and rodents, compared with that of males, indicating a sex bias.

To elucidate whether the sex bias in 5-LO activity influences burn wound healing, we generated global Alox5 (Arachidonate 5-lipoxygenase, the gene that encodes for 5-LO) knockout mice (Alox5−/−) in C57BL/6 background. Alox5−/− and littermate Alox5+/+ control mice underwent burn injury, and wound tissues were isolated and examined 3, 7, and 14 days later.

Our data show a sex-specific effect of 5-LO activity in burn wound healing. Female Alox5−/− mice exhibited increased re-epithelialization compared to their controls, on day 14 post injury (pi). Furthermore, flow cytometric analysis on day 7 pi revealed increased total numbers of wound immune (CD45+) cells, but no differences in their make-up, in female Alox5−/− mice compared to their controls. This suggests that 5-LO activity regulates overall immune cell recruitment in burn wounds of female mice; however, whether these cells are directly involved in the wound healing process is not currently elucidated.

On the other hand, Alox5−/− male mice showed decreased re-epithelization on days 7 and 14 pi, compared to their controls. In contrast with female mice, infiltration levels of male Alox5−/− wounds on day 7 pi were not different than those of their controls. This suggests that the observed delay in re-epithelialization may be independent of the inflammatory environment and could instead be mediated via 5-LO-induced effects on local skin cells.

Collectively, these results suggest that 5-LO activity affects burn wound healing in a sex-dependent manner. We hypothesize that sex-specific 5-LO-induced changes in the eicosanoid profile play a key role in the differential burn wound healing process. In this study, we will present eicosanoid wound profiles over time and in relation to sex and genotype. Differences in collagen production and transcriptome signatures will also be presented. Our study will help elucidate sex-specific 5-LO evoked mechanisms in burn wound healing.

P4.02 | Angiography: A Limb Salvage Strategy Following Lower Extremity Thermal Injury In Patients With Diabetes Mellitus And Peripheral Artery Disease

Desiree Pinto1, Isabel Snee1, Saher Sabri2, Lauren Moffatt1, Taryn Travis1, Jeffrey W. Shupp2, Shawn Tejiram1
1Firefighter’s Burn and Surgical Research Laboratory, Washington; 2The Burn Center, MedStar Washington Hospital Center, Washington, DC

Background: Patients with diabetes mellitus (DM) or peripheral arterial disease (PAD) are at high risk of wound complications and amputations following lower extremity thermal injury. Algorithms to improve healing and limb salvage in chronic wounds from DM or PAD include angiography to facilitate peripheral blood flow analysis and revascularization. However, the role of angiography in patients with acute thermal injuries and DM or PAD have yet to be elucidated. This study describes a regional burn center’s experience incorporating angiography into the acute management of lower extremity thermal injuries in patients with DM or PAD.

Methods: Patients admitted with a lower extremity partial or full thickness thermal injury and history of DM or PAD between 2021 and 2023 were retrospectively reviewed. Patients with an abnormal arterial-brachial index (ABI), which prompted angiography evaluation, were included. Vascular disease identified and interventions performed during angiography were obtained. Clinical outcomes, including graft loss and amputations were evaluated.

Results: There were 23 patients with a lower extremity thermal injury, history of DM or PAD, and an abnormal ABI that underwent lower extremity angiography. Most patients were male (65.2%), had a thermal injury to their foot (82.6%) and had uncontrolled DM (median hemoglobin A1c of 10.2 mmol/mol). The median total body surface area (TBSA) was 2.0%. Vascular disease was identified in 18 patients (79%) by angiography. The tibial arteries were the most common site of vascular disease (87%), followed by the popliteal (14%) and superficial femoral arteries (14%). Incomplete pedal arch was identified in 5 patients (22%), with microvascular disease as the primary cause (60%). Vascular disease was amendable to re-vascularization attempts using balloon angioplasty in 9 patients (50%), with an 88.8 success rate. After re-vascularization over half of patients (55.6%) healed without grafting, while most patients without re-vascularization...
required further grafting (71.4%). When grafting was required, the rate of graft loss necessitating prolonged wound care or re-operation was less for patients who underwent re-vascularization (7.1% vs 14.3%). Amputations were necessary in 33.3% of patients who underwent re-vascularization compared to 14.3% of patients who did not have re-vascularization. There is not enough power to reach statistical significance.

Conclusions: This study is the first to describe the role of angiography in the acute management of lower extremity thermal injuries in patients with DM or PAD. We demonstrated angiography’s ability to improve vascularization in the lower extremity to promote wound healing without grafting and minimize graft loss. Further studies are needed to better understand the impact of angiography on limb salvage.

P4.03 | Evaluation Of Subcutaneous Combination Ibuprofen And Resolin D2 Therapy To Mitigate Burn Progression

Marc Thompson1, Sergio García2, Lucy Shaffer3, Michelle Holik2, David Larson1, Logan Leatherman1, Valeta Sanders2, Anna Ochoa1, LTC Julie Rizzo2, Robert Christy1, Christine Kowalczewski2
1US Army Institute of Surgical Research, JBSA Ft Sam Houston, TX; 2Trauma Surgery, Brooke Army Medical Center, JBSA Ft Sam Houston, TX

Introduction: Thermal injuries are common to all military conflicts. Difficulties treating partial-thickness burns in the field are further complicated by their propensity to progress/converge to deep-partial or full-thickness burns within the first 24 hours of injury. It is hypothesized that burn conversion (deepening) occurs as a function of irregular perfusion linked to inflammation, which ultimately causes progressive necrosis in the wound. This study hypothesizes that subcutaneous administration of inflammation mitigating Ibuprofen and Resolvin D2, in a porcine model of burn progression, will prevent partial-thickness thermal burns from progressing to deep partial/full-thickness injuries, potentially improving wound healing outcomes.

Materials and Methods: To create partial-thickness burns in isoflurane anesthetized Yorkshire swine (also provided Buprenorphine SR for pain management), a 100-C heated 6 cm diameter brass cylinder was applied to the dorsum, to create ten burns per animal. An N of 6 animals were used; all 6 received the following treatments (2 wounds per treatment/animal): 1. Gauze (standard of care control), 2. Saline (vehicle control) 3. Ibuprofen (0.1 mg/mL) 4. Resolin (0.05 mg/mL) 5. Combination treatment of Ibuprofen and Resolvin D2 at the same doses. Treatments were injected 3 hours post-injury and once again 24 hours later to simulate a prolonged field care (PFC) scenario. Before the burn injury and at pre-assigned time points post-burn (days 0, 1, 3, 7, 14, 21, and 28), wounds were imaged (Digital, Silhouette, Molecular Light, Moor Laser Doppler Imaging) and biopsies harvested. Half of the biopsy was evaluated histologically by H&E and immunohistochemistry (IHC) to determine the depth (normalized % of dermal depth) of damaged tissue and wound healing over the experimental period. Remaining biopsy samples were preserved for proteomic analysis.

Results: A one-way Analysis of Variance (ANOVA) was employed to determine that, 24 hours after injury, combination therapies of Ibuprofen and Resolvin D2 prevented burns from progressing to deep partial-thickness burns (55.8 ±21.4%) (p<0.0001), whereas the military standard of care (Gauze) did not (66.6 ±21.9%). In addition, all experimental treatments reduced the lateral size (surface area) of burn injuries compared to Gauze, albeit not to a statistically significant degree.

Conclusions: Therapies already available in the field (Ibuprofen) and those less so (Resolvin D2) have previously displayed the ability to mitigate injury severity by lessening the degree of inflammation. We have shown here that while individual therapies may improve wound healing outcomes compared to the current standard of care, it may be the case that therapies used in concert, delivered directly to the injury, with varying mechanisms of action, can provide even greater efficacy.

P4.04 | ELU42, A Small Molecule WNT Signaling Inhibitor, Significantly Accelerates Wound Closure And Promotes Regenerative Repair Following Cutaneous And Third-Degree Burn Injury In Yorkshire Pigs

Daniel Holsworth, Sarika Saraswati, John Delgado, Michael Stone
Eluciderm Inc, San Diego, CA

BACKGROUND: The canonical WNT signaling pathway is quiescent in many mammalian organs and becomes activated in response to injury. WNT signaling promotes fibrotic wound healing (including scarring) following acute cutaneous injury. Topical “spray-on” application of a proprietary WNT signaling inhibitor accelerated wound closure and promoted regenerative cutaneous repair in acute and third-degree burn wounds.

METHODS: In this study, we utilized two (2) porcine models to analyze wound repair. Six (6) full-thickness 3 x 3 cm2 acute and ten (10) third-degree burn excisional wounds were created on the backs of Yorkshire pigs. ELU42, a novel, potent, aqueously soluble, topical “spray-on” small molecule WNT signaling inhibitor, was applied three (3) days a week (Mon, Wed, Fri; 200mL) up to Day 30. The animals were allowed to heal for another 30 days before being sacrificed at Day 60. Histopathological analyses were performed on excised tissues.

RESULTS: In full-thickness acute and third-degree excisional wounds, topical application of the novel small molecule WNT signaling inhibitor, ELU42, significantly promoted wound closure, and also promoted regeneration of tissue, as evidenced by the presence of restored skin architecture with adnexal structures and restoration of well-organized granulation tissue. A statistically significant increase in rete peg formation at the dermal–epidermal junction was also identified. Significance calculations were performed using a two-way Analysis of Variances (ANOVA) using the Prism10 software (Graph-pad prism). P<0.05 was considered significant.
CONCLUSIONS: Until now, studies using small molecule WNT signaling inhibitors were limited for therapeutic usage due to their poor aqueous solubility. We have created ELU42, a water-soluble small molecule WNT signaling inhibitor, in spray-on form. It is a non-toxic potential drug, and does not require a sterile environment when applying to traumatic soft tissue (acute) wounds and third-degree burns. Our study presents a stable, potent, bioavailable, small molecule WNT signaling inhibitor that has strong pharmacological potential for use as a therapeutic for the regenerative repair of cutaneous wounds.

P4.05 | The Use Of Therapeutics Peptides With Full-Thickness Skin Columns To Improve Healing Of Excisional Wounds

Kristo Nuutila1, Anders Carlson2, Sean Christy2, David Larson2, Chan Rodney2, Thomas Darling3, Ira M. Herman4
1USAISR, San Antonio, TX; 2The Metis Foundation, San Antonio, TX; 3SUHS, Bethesda, MD; 4TUFTS University, Boston, MA

Introduction: In split thickness skin grafting (STSG) a very thin layer of skin containing the epidermis and a small portion of the dermis is grafted. This is done to create a quick coverage on the receiving site but also to minimize donor site morbidity. One of the drawbacks is the absence of adnexal structures and thus the grafted wounds lack basic skin functions such as durability, thermoregulation, maintenance of hydration and lubrication. An alternative technique to solve this problem is fractional autologous skin grafting using full-thickness skin columns (FTSC). Harvesting occurs orthogonally by taking numerous individual skin columns containing the epidermis down through the dermis including the skin appendages. Bioactive peptides have been shown to rescue hair follicular unit survivals. The purpose of this study is to investigate whether transplantation of FTSCs in combination with bioactive peptides would better reconstitute skin function due to the positive effect of the peptides on adnexal structures within FTSCs.

Materials and Methods: Up to 16 standardized full-thickness excisional wounds were created on the dorsum of two anesthetized pigs. Analgesia was provided prior to all surgical procedures and the animals were monitored for pain twice every 24 hours for the first 72 hours. FTSC biopsies were harvested from donor sites located on the cranial-dorsum at a ratio of up to sixteen 1.5 mm-diameter skin columns/1cm². The wounds were randomized to receive either FTSC + bioactive peptide hydrogel (carboxymethyl cellulose), FTSC + scrambled peptide hydrogel, FTSC only or left untreated. Healing was monitored for up to 28 days. The wounds were excised and fixed in formalin for histologic analyses. In addition, non-invasive imaging systems were utilized to assess both wound healing and quality of healing.

Results: By day 14, the FTSC + bioactive peptide, FTSC + scrambled peptide, FTSC only and untreated wounds were 71%, 55%, 88% and 27% re-epithelialized respectively. The FTSC + bioactive peptide and the FTSC only treated wounds were significantly more re-epithelialized than the untreated wounds (p < 0.05). By day 28 all the FTSC transplanted wounds were fully re-epithelialized and the difference to the untreated wounds was statistically significant (p < 0.05). The results demonstrated that wound treated with the FTSC + bioactive peptide had more mature epidermis at day 28 post transplantation. The FTSC + bioactive peptide treated wounds had significantly more rete ridges and more mature epidermis in comparison to the other groups. In terms of wound contraction, no significant differences were observed.

Conclusions: FTSC can be transplanted in a hydrogel to close a full-thickness wound. Furthermore, it was shown that transplantation of FTSC in combination with bioactive peptides increased quality of wound healing.

P4.06 | Immediate Application Of Topical Anti-Inflammatory Agents On Burn Wounds And Their Effect On Healing

Jamie Neelon1, Irene Yau2, Kristo Nuutila3
1General Surgery, Brooke Army Medical Center, San Antonio, TX; 2General Surgery, William Beaumont Army Medical Center, El Paso, TX; 3USAISR, San Antonio, TX

Introduction: Though recent advances in the treatment of burns have considerably improved overall survival rates, they have also highlighted several long-term sequelae related to the injury. Hypertrophic scars, for example, can impair function, reduce quality of life, and require multiple procedures as well as physical therapy. The purpose of this study was to investigate the effects of topical application of anti-inflammatory drugs on burn wound progression, overall wound healing, and quality of healing.

Materials and Methods: 15 deep-partial thickness burns were created on the dorsum of four anesthetized swine using a custom burn device at 100°C. Analgesia was provided prior to all surgical procedures with buprenorphine and the animals were monitored for pain twice every 24 hours for the first 72 hours. The burn wounds were randomized to receive amiloride, celecoxib, dexamethasone or minocycline formulated in a hydrogel. Silver sulfadiazine cream and blank hydrogel acted as controls. The animals were followed for 90 days and the wounds were assessed on days 3, 14, 28 and 90 post-burn. Assessments were performed using digital photographs (macroscopic healing, contraction), laser-Speckle imagery (blood perfusion), 3D camera (scarring, pigmentation), and histology (burn wound depth, epidermal thickness, rete ridges).

Results: 15 deep-partial thickness burns were evaluated. It was shown that on day 3, burn depth varied from 155 μm (celecoxib) to 222 μm (blank hydrogel) but no statistically significant differences were observed. In terms of wound healing, the results showed that by day 14 post-burn, percent wound closure ranged from 45% (dexamethasone) to 84% (celecoxib) but no significant differences were observed. By day 28 post-burn all the wounds were fully healed. Quality of healing was studied on day 90 post-burn. Wound contraction varied from 28% (celecoxib) to 43% (minocycline) but no significant differences...
were seen. No differences were observed in the thickness of epidermis or number of rete ridges.

Conclusions: This study concluded that topical application of amiloride, celecoxib, dexamethasone or minocycline formulated in a hydrogel did not mitigate burn wound progression, promote wound healing or increase quality of healing when compared to controls.

2024 WHS Display Poster Abstracts

P14 | Using Bioactive Hydrogel Dressing to Prevent Post-Burn Scarring

Fateme Fayyazbaksh1, Yue-Wern Huang2, Ming Leu1
1Mechanical and Aerospace Engineering, Missouri University of Science and Technology, Rolla, MO; 2Biological Sciences, Missouri University of Science and Technology, Rolla, MO

Recent advances in 3D bioprinting have led to the development of innovative solutions for wound healing. Among various advanced dressings, hydrogel materials have gained significant attention for burn wound treatment in clinical practice. Advances in acute burn wound care have significantly decreased mortality rate in patients with severe burns. However, up to 70% of burn victims develop post-burn hypertrophic scars. In this study, 3D-printed dressings were fabricated with an extrusion-based bioprinter using bioinks consisting of gelatin, alginate, and bioactive borate glass (BBG). After ion crosslinking, the 3D-printed bioactive hydrogel dressings were characterized and exhibited Young's modulus in the range of normal skin, with 10-day stability in phosphate buffer saline. The hydration activity of bioactive hydrogel dressings were measured and compared to the FDA-approved commercial products. Over the course of 10 days, the bioactive hydrogels dressings steadily released 74% of their entrapped water, while the commercial products released their entire water content within 24 hours. The preclinical safety and efficacy of the dressings were investigated using a porcine model with a second-degree burn wound for 70 days. Our findings showed that the 3D-printed dressings with BBG exhibited faster wound closure with minimal scarring. Histological analysis suggested that 3D-printed dressings with BBG developed more uniform re-epithelialization and tissue remodeling compared to the FDA-approved commercial products. Our immunohistochemistry analysis revealed that BBG improved early angiogenesis and maturation of blood vessel in later stages of wound healing, which is associated with the therapeutic ions released from BBG particulates. After 70 days, the deposition of collagen fibers was measured at 95 ± 16 μm and 278 ± 74 μm with 21% and 10% basket-weave patterns, in the bioactive hydrogel dressing and commercial product, respectively. Finer collagen fibers, 27% decrease in ki67 and 36% increase in regeneration of hair follicles confirmed the enhanced scar preventing outcomes in bioactive hydrogel dressing compared to the commercial product. This can be attributed to the bioactive formulation and the 3D-printed porous surface, which promote a moist wound healing environment, thereby allowing for normal wound remodeling. Findings from this research provide valuable knowledge that advances bioprinting techniques in wound healing applications, specifically in the development of bioactive formulations for scarless wound healing.

P15 | Subcutaneous Adipose Protein Expression Ratios of PPARgamma/HIF-1alpha Predicts Healing Outcome In Obesity Wound

Ji LIN1, Xiao-ning GAO2
1Department of Basic Medicine, Graduate School, Chinese PLA General Hospital, Beijing, China; 2Department of Hematology, Fifth Medical Center, Chinese PLA General Hospital, Beijing, China

BACKGROUND: Healing in obesity wounds is impeded for indiscernible mechanism and lack of predictive indicators. Epidermal stem cells (EpiSCs) and adipocytes are important components in skin structure, metabolism and healing, while adipogenesis and adipocyte differentiation have been verified crucial for wound healing. As PPARgamma and HIF-1alpha are vital transcriptional factors balancing in adipogenesis and differentiation, we hypothesize that subcutaneous adipose protein expression ratios of PPARgamma/HIF-1alpha (P/H ratio) may reflect EpiSCs-adipocytes interaction and predict healing outcome in obesity wound.

METHODS: A high fat-diet-induced obesity model using Sprague-Dawley rats was established, setting groups of sham-injury (Sham), injury without cell transplantation (Injury), and injury plus EpiSCS transplantation, then a 6-mm diameter full-thickness excision was developed on the dorsal skin. 1×10⁶ epidermal basal stem cells isolated from neonatal mice skin and suspended in 30 μl 1×PBS were injected subcutaneously into each wound in EpiSCs group, as equivalent 1×PBS was injected similarly in Sham and Injury groups. Skin wounds were harvested and subjected to histological investigations.

RESULTS: Ameliorative histological healing and PerilipinA(+) adipocytes recruitment were found in EpiSCs group at 1, 3, 7 and 14 days after injury. Subcutaneous adipose P/H ratio determined by immunohistochemistry in EpiSCs group was decreased at 1 day but increased at 7 day (P<0.05, vs Injury), and it remained no significant difference yet a rising trend at 3 or 14 days (P>0.05, vs Sham or Injury). Subcutaneous adipose P/H ratio in Injury group was decreased at 7 day (P<0.05, vs Sham), and remained no significant difference with trends to rise at 1 or 3 days but decrease at 14 day (P>0.05, vs Sham).

CONCLUSION: EpiSCs may resume PPARgamma/HIF-1alpha-balanced subcutaneous adipogenesis and adipocyte differentiation to promote healing in obesity.

P16 | Wound Healing Effects Of Paste Type Acellular Dermal Matrix Subcutaneous Injection

Joon Hyuk Lee
Plastic surgery, Yeung Nam University, South Korea
**Background:** Acellular dermal matrix (ADM) helps wound healing by stimulating angiogenesis, acting as a chemotactic agent for endothelial cells, providing growth factors, and permitting a substrate for fibroblasts to attach. The current standard for using paste-type ADM (CG Paste) in wound healing is direct application over the wounds. The major concerns regarding this method are unpredictable separation from the wounds and absorption into negative-pressure wound therapy devices. This study aimed to investigate the effects of subcutaneous injection of paste-type ADM on wound healing in rats.

**Methods:** Full-thickness skin defects were created on the dorsal skin of rats. Eighteen rats were randomly divided into three groups and treated using different wound coverage methods: group A, with a saline dressing; group B, standard application of CG Paste; and group C, injection of CG Paste. On postoperative days 3, 5, 7, 10, and 14, the wound areas were analyzed morphologically. Histological and immunohistochemical tissue analyses were performed on postoperative days 3 and 7.

**Results:** Groups B and C had significantly less raw surface than group A on postoperative days 10 and 14. Collagen fiber deposition and microvessel density were significantly higher in group C than in groups A and B on postoperative days 3 and 7.

**Conclusions:** This study showed comparable effectiveness between subcutaneous injection and the conventional dressing method of paste-type ADM. Moreover, the injection of CG Paste led to improved wound healing quality through the accumulation of collagen fibers and an increase in microvessel density.

**P18 | Rapid Fabrication Of Polyvinyl Alcohol Hydrogel Foams With Encapsulated Mesenchymal Stem Cells For Chronic Wound Treatment**

Nghia Thai, Emily Fittante, Mary Beth Monroe

*Biomedical and Chemical Engineering, BiolInspired Syracuse, Syracuse University, Syracuse, NY*

**Purpose:** The purpose of this study is to develop a tunable hydrogel platform with encapsulated mesenchymal stem cells to enhance chronic wound treatment.

**Methods:** We synthesized a thiol methacrylate polyvinyl alcohol (TPVAMA) hydrogel system based on the thiol-ene reaction between methacrylated PVA (PVAMA) and thiolated PVA (TPVA). We introduced porous structures into the hydrogels via a gas-blowing process to improve nutrient transfer and waste removal, which could support cell viability. In this process, sodium bicarbonate and citric acid were combined into the system to create carbon dioxide, which was trapped during hydrogel fabrication to form a porous structure. Also, methacrylated gelatin (GelMA) was added into the system to improve cell attachment. Cells were mixed into hydrogel solutions prior to fabrication to obtain porous, cell-laden hydrogels. We obtained a library of hydrogel samples to utilize for further characterization.

**Results:** Cytocompatibility tests showed that all TPVAMA hydrogels have cell viability >95%, demonstrating this system is suitable for cell growth. 3D microscale images exhibited that we successfully encapsulated cells into hydrogels with varied dimensions. Viability of...
encapsulated A375 cells was measured over 14 days using a Live/Dead assay. At 14 days, viability was significantly higher in porous hydrogels with GelMA (porous-TPVAGelMA) than corollary non-porous hydrogels with TPVAGelMA and without GelMA (TPVAMA), with viabilities of 95±3%, 84±8%, and 71±18%, respectively (n=3, p < 0.05). In the encapsulation of fluorecently labeled 3T3 fibroblasts, porous-TPVAGelMA showed a significant increase in the number of cells and cell spreading over 14 days compared with non-porous TPVAGelMA and TPVAMA controls. These results indicate that GelMA enhances cell proliferation and attachment, while porous structures aid in maintaining long-term cell viability. In degradation studies, hydrogels containing higher TPVA content degraded faster in hydrolytic and oxidative solutions. Thus, the degradation rate can be tuned based on the PVAMA and TPVA ratio, indicating that the hydrogel system could be designed to degrade after implantation while supporting healing.

**Conclusion:** These studies demonstrate that TPVAMA hydrogel foams are a potential platform for chronic wound dressings that are degradable, tunable, and suitable for cell encapsulation. Current work is focused on performing scratch assays and ex vivo pig skin experiments with encapsulated mesenchymal stem cell samples to observe the effects of the hydrogel system on healing processes.

**P19 | Fluoxetine Delivery Through An Integrated Bioelectronic Device Promotes Wound Healing In Swine**

Hsin-ya Yang1, Houpu Li2, Wan-Shen Hee2, Prabhat Baniya2, Guillermo Villa-Martinez1, Anthony Gallegos1, Kan Zhu2, Cynthia Recendez2, Moyasar A. Alhamo1, Narges Asefifeyzabadi2, Tiffany Nguyen2, Maryam Tebyani2, Gordon H. Keller2, Alexie Barbee2, Ansel Trevino2, Athena Soulika1, Sydnie Figurres2, Mircea Teodorescu2, Min Zhao2, Rivkah Isseroff1, Marco Rolandi2

1Dermatology, University of California, Davis, Sacramento, CA; 2Electrical and Computer Engineering, University of California, Santa Cruz, Santa Cruz, CA; 3Ophthalmology, University of California, Davis, Davis, CA

Epidermal wound healing, including hemostasis, inflammation, cell proliferation, cell migration, and tissue remodeling, is a highly coordinated biological process. To optimally promote wound closure by a temporally and spatially controlled drug delivery system, a team of bio- and electrical engineers, computer scientists and wound specialists collaborated to create this iontophoresis bandage device with an actuator for drug delivery, and a sensor for wound monitoring. Previously we have demonstrated that this bioengineered device with programmable bioelectronic ion pumps to release protonized fluoxetine in negligible amount of solution, can increase re-epithelialization and decrease macrophage M1/M2 ratio on mouse wounds. Here we further integrated an actuator, the fluoxetine iontophoresis bandage, and a sensor, a microscopic camera with LED lights, onto a single platform with on-board PCB electronics and wireless communication on the device for the in vivo swine tests. Full-thickness, 20mm circular wounds were created on the back of Yorkshire pigs and the fluoxetine device with camera was applied to the wounds. Fluoxetine was targeted to deliver 0.45mg/wound/day during the daily delivery program. On the post-operative days 3 or 7, wound tissue was harvested to examine healing. On day 7, the treatment with fluoxetine device showed a trend of improved re-epithelialization by 50.4% compared to standard of care (n=6-8 wounds, p=0.056). The anti-inflammatory macrophage M2 subtype also increased with the fluoxetine device treatment, which results in decreased ratios of M1/M2 by 77.0% on day 3 and by 62.5% on day 7. Another indication of the tissue repair is innervation to the wound site. By using the expression of MAP2, Microtubule-Associated Protein 2, as a surrogate marker for neuronal ingrowth and dendrite extension into the wound, we demonstrated that the relative gene expression of MAP2 for fluoxetine-treated wounds on day 7 was 0.9 compared to 0.2 for the control, a 4.5-fold increase on wound edge. The integrated bioelectronic device with fluoxetine delivery has a great potential for wound treatment by reducing the burden for daily drug application, and possibly increasing patients’ compliance. It also demonstrates that the fluoxetine released from the device retains its reparative biological activity to promote healing. In the future, we hope to further optimize the device design for the next stage of development for device commercialization and clinical use.

**P20 | Optimization Of Real-Time Qpcr For Measuring Inflammation Index In Diabetic Foot Ulcer Wounds In Model Tissue Samples**

Juan Cortes-Troncoso, Yveeka Marcellus, Aliyah Stephens, Kara L. Spiller

School of Biomedical Engineering, Science and Health Systems, Drexel University, Philadelphia, PA

Inflammation and wound healing are complex, linked processes that are alternated in nonhealing diabetic foot ulcers (DFU). Our research has shown that while initial pro-inflammatory activation of immune cells is critical for the initiation of wound healing processes, prolonged activation directly impairs it. After recognizing that transition from the early inflammatory to the late resolution phase is required for successful healing, we developed a composite biomarker using the ratio of 4 early-stage pro-inflammatory gene markers to 3 late-stage inflammation-resolution biomarkers, referred to as the Inflammation Index. The Inflammation Index is an indirect measurement of the wound’s healing stage. Our previous studies measured the Inflammation Index via RT-qPCR using RNA extracted from debrided wound tissue, suggesting that this score might have the potential to identify those wounds that are more likely to respond to conservative treatment versus those that may benefit from a more aggressive approach. To evaluate the expression of biomarkers that comprise the Inflammation Index, quality RNA is essential. The chronic wound environment is particularly damaging for RNA because of its high levels of enzymes and cellular debris containing RNases. Therefore, our goal in this project was to optimize biomarker detection and determine the minimum
sample quality and quantity in which the Inflammation Index can be reliably detected using RT-qPCR.

By using an experimental model of intact and partially degraded RNA from in vitro-cultivated macrophages derived from human primary monocytes, we proved how quality control (QC) metrics affect biomarker expression. We determined that degradation-influenced shifts of threshold values (Ct-values) can be compensated by calculating delta-Ct values between test genes and the mean values of several control genes. We demonstrated that the Inflammation Index can be measured on samples with even low-quality and quantity RNA. Additionally, we validated how sample storage/shipping conditions affect RNA QC metrics.

Based on these results, we conclude that by using controllably degraded cell samples in vitro to model damaged tissue, the measurement of the Inflammation Index in DFU samples was appropriately optimized. From a translational perspective, these results validate the biomarker detection method (RT-qPCR) and determine the minimum QC metrics that must be satisfied for the biomarkers to be reliably measured, using real-world samples collected from the Diabetic Foot Consortium (DFC). By using samples from the DFC, we will measure the Inflammation Index’s ability to predict healing in response to the standard of care, in order to ultimately personalize treatment for patients with hard-to-heal ulcers and to refine entry criteria for clinical trials of new treatments.

Results/Discussion: Four out of nine tapes (n=10) showed a statistically significant similarity between mean peel forces on the skin mimic vs human skin. The remaining did not exhibit similarity, primarily due to the large variability of the clinical data vs the high repeatability of the skin mimic data.

Conclusion: The skin mimic showed a correlation with the data from the clinical study on human skin, but with a higher level of repeatability as indicated by the low standard deviation of skin mimic data. A high level of repeatability will be critical when evaluating adhesives for changes in performance, as it becomes increasingly more difficult to attribute differences in results to variations in the adhesive when variability is high due to the method and/or study participants.
were P. aeruginosa, S. aureus, and E. coli. Negative cultures were found in 22% of closed wounds and 13% of open wounds.

Conclusion: Our findings suggest that BMI may be correlated with early wound status and the incidence of postoperative complications, while osteomyelitis status may not. Future studies should further evaluate the effect of BMI on pressure ulcer associated complications. This may further guide preoperative planning and patient expectations.

P23 | Dermal Fibroblasts Contribute to Oxidative Stress in Diabetes

Bailey D. Lyttle\textsuperscript{1}, Hanan Elajaili\textsuperscript{1}, Eva S. Nozik\textsuperscript{1}, James R. Bardill\textsuperscript{1}, Carlos Zgheib\textsuperscript{2}, Kenneth W. Liechty\textsuperscript{2}

\textsuperscript{1}University of Colorado, Denver, CO; \textsuperscript{2}Surgery, University of Arizona, Tucson, AZ

Background: Diabetes is a common medical condition with numerous comorbidities including chronic wounds. Impaired wound healing in diabetes has been associated with inflammation and oxidative stress secondary to reactive oxygen species (ROS), which have traditionally been measured by evaluating dysregulation in enzymes that produce ROS. ROS also act as second messengers for cell populations present within diabetic wounds to stimulate further inflammation, resulting in a cycle of chronic inflammation and oxidative stress. Fibroblasts are a key cellular population involved in wound healing and have historically been identified to play a role in extracellular matrix formation, inflammation, and angiogenesis. Given that ROS interact with other cell populations beyond inflammatory cells, we theorized that fibroblasts may interact with ROS beyond their known cellular function and contribute to the overall milieu of the wound. We therefore hypothesized that both whole blood and dermal fibroblasts isolated from diabetic mice would demonstrate increased ROS production as measured by electron paramagnetic resonance (EPR), which allows for direct measurement of ROS rather than just enzymatic markers.

Methods: Fibroblasts were isolated from skin of 12-week-old female diabetic (db/db) and heterozygous (db/+\textsuperscript{1}) control mice (N=1 per group) and cultured in low-glucose Dulbecco’s Modified Eagle Medium (DMEM) with 10% fetal bovine serum (FBS) and 1% antibiotic-antimycotic in tissue culture flasks. Once confluence was achieved, fibroblasts were passed into 6-well plates (N=6 per group) and cultured until 80% confluence was reached. Whole blood was extracted from 12-week-old female diabetic and heterozygous control mice (N=2 per group). Production of ROS was evaluated with electron paramagnetic resonance (EPR) spectroscopy using a hydroxylamine probe that upon the reaction with ROS generates a nitroxide radical that can be detected by EPR. Nitroxide levels generated from both whole blood and cultured fibroblasts treated with the EPR probe and normalized to protein concentration were compared between diabetic mice and heterozygous controls.

Results: Fibroblasts from diabetic mice demonstrated significantly higher ROS production compared to heterozygous controls (p=0.042). Similarly, whole blood from diabetic mice demonstrated significantly higher ROS production compared to heterozygous controls (p=0.047).

Conclusions: ROS production was significantly elevated in dermal fibroblasts of diabetic mice compared to controls, suggesting that fibroblasts may play a role in the production of ROS that leads to oxidative stress. Additionally, whole blood of diabetic mice demonstrated upregulation of ROS, suggesting the presence of a systemic effect. Dermal fibroblasts remain a key regulator of impaired wound healing in diabetes due to multiple mechanisms and may serve as a target for potential therapeutics.

P24 | Effect Of Pre-Operative Wound Characteristics And Previous Treatments On Pressure Ulcer Healing Outcomes And Disposition

Kirtana Sandepudi, Robert Galiano

Department of Plastic and Reconstructive Surgery, Northwestern University Feinberg School of Medicine, Chicago, IL

Background: Pressure ulcers are injuries to the skin and underlying tissue that often develop into chronic wounds and can significantly impact quality of life. After surgical closure, many patients experience complications such as dehiscence, maceration, drainage, and necrosis, and may require long-term rehabilitation to achieve functional recovery. The objective of this study is to evaluate the association between wound characteristics prior to surgical closure and wound healing outcomes including complication rates and disposition.

Methods: In this prospective study, patients with grade III/IV buttock pressure ulcers were admitted for surgical closure and observed postoperatively for at least 14 days. Wound characteristics upon admission, such as wound location, size of wound (length, width, depth), and previous wound treatment (previous closure, debridement, negative pressure wound therapy, hyperbaric oxygen, biologics, revascularization) were recorded. Wound volume was estimated by multiplying length, width and depth. Outcome measures included 2-week complication rates, length of hospital stay, disposition, and wound closure rates at 2 weeks, 1 month, 6 months, and 12 months. Odds ratios and t-tests were used to analyze outcomes.

Results: Of a total of 68 patients who completed the study, 36 (53%) had ischial wounds, 25 (37%) had sacral wounds, and 7 had wounds in other locations. 11% of patients with ischial wounds experienced complications, while 44% of those with sacral wounds experienced complications (OR=0.16, 95% CI 0.04-0.59). The average number of complications per patient was 0.19 in patients with ischial wounds and 0.67 in patients with sacral wounds (p = 0.01). Patients with sacral wounds also had a longer average hospital stay than those with ischial wounds, by 1.7 days (p = 0.04). Increased wound volume was associated with lower chance of closure at 14 days (p = 0.006), but did not have an effect on closure rates at long-term follow-ups.
Increased wound length was also independently associated with lower chance of closure on day 14, although this result only approached significance ($p = 0.056$). Patients who did not receive any previous treatments were more likely to be discharged to a long-term care facility than patients who had previous treatment (OR=6.22, 95% CI 1.07-36.2). The average number of complications per patient was 0.09 for patients who had previous wound closure and 0.42 for patients who had not had previous closure ($p = 0.01$).

**Conclusion:** Overall, this study found that wound characteristics prior to surgical closure, including size, location, and previous treatments, have a significant impact on post-operative complication rates and disposition in patients with grade III/IV buttock pressure ulcers. These findings warrant further investigation and can help guide management and pre-operative counseling.

**P25 | A Novel Ex Vivo Model Of Peristomal Skin Damage For Development Of Ostomy Adhesives**

Jayant Joshi$^1$, Tage Carlson$^1$, Abram D. Janis$^1$, Hallie Goldstein$^2$, Bethany Perez-White$^2$

$^1$Hollister Incorporated, Libertyville, IL; $^2$Dermatology, Feinberg School of Medicine, Northwestern University, Chicago, IL

**BACKGROUND.** Ostomy surgery is often the only effective treatment for diseases affecting normal digestive or urinary function. The resulting stoma, or opening, in the abdomen is affixed with a waste collection pouch that is secured to the surrounding skin with adhesive tape. Ostomy pouches are Class I medical devices and must not only adhere to the skin and protect it from body waste, but also be amenable to routine removal and re-application for the duration of the treatment. Repeated removal of adhesives and exposure to bodily wastes from the stoma (dejecta) causes peristomal skin damage that can lead to complications and reduced quality of life.

**METHODS & RESULTS.** Our goal is to develop novel adhesive formulations that can a) adhere for extended periods of time; b) be removed with minimal skin trauma; and c) minimize the impact of stoma leakage. Human clinical modeling of peristomal skin irritation is costly and difficult to achieve in healthy volunteers, therefore clinical models are challenging as a high-throughput screening tool for formulation development. Therefore, to achieve our goal we developed an ex vivo model of peristomal skin using de-identified, discarded abdominoplasty tissues to identify strong novel adhesive formulation candidates to move into clinical models. Our current objectives are to understand the effects of repeated tape-stripping with 2 different hydrocolloid adhesive formulations and the damage caused to skin by a mixture of digestive enzymes. Morphological analysis of H&E-stained cross sections of tape-stripped tissue (applied and removed five times) revealed distinct differences in the extent of damage to the stratum corneum, ranging from no damage to complete loss with exposure of the stratum granulosum. Transepidermal water loss (TEWL) readings taken immediately after each stripping event correlated with observed epidermal damage. These TEWL results align with results from prior clinical studies of these adhesives. To understand the skin damage resulting from exposure to digestive enzymes in dejecta, we topically applied a cocktail of trypsin, chymotrypsin, and elastase for 1 h to skin tissue. Enzymatic exposure caused significant decapsulation of the stratum corneum and ablation of the stratum granulosum, as determined by H&E staining. Moreover, the enzymes significantly reduced the immunofluorescent expression of epidermal barrier proteins filaggrin and loricrin.

**CONCLUSIONS.** Taken together, these results show significant mechanical and enzymatic epidermal barrier damage and modeling of peristomal conditions. Moving forward, we will combine tape-stripping with enzyme exposure to more closely model the combination of factors affecting the peristomal skin and characterize additional adhesive formulations with improved skin protective capabilities.

**P26 | Finite Element Analysis Modeling And Preclinical Study To Assess The Tissue Strains And Granulation During Negative Pressure Wound Therapy With Instillation Using A Felted Foam With 10mm Holes**

Amy K. McNulty, Robert Wilkes, Brenda Marchand, Shannon Ingram, James Sieracki

MSD, 3M Company, St. Paul, MN

**Background:** Not all patients with wounds are able to undergo surgical debridement. Negative pressure wound therapy with instillation (NPWTi) using a felted, reticulated open cell foam with an array of 10 mm holes (VCC) has been used in clinic with NPWTi to eliminate non-viable material from the wound bed$^1$. The current study uses Finite Element Modelling (FEA) to elaborate the biomechanical stresses, strains and work imparted to tissue with VCC versus the same felted foam without through holes (VC). A porcine study was conducted to compare theoretical FEA results with those in actual sloughy wounds.

**Methods:** For this FEA model, skin was modeled as Neo-Hookean with a Young’s Modulus of 0.05 MPa and Poisson’s ratio of 0.15. Foam samples were compressed between steel platens to 70% compression to generate stress strain curves used in the model. For the preclinical study, full-thickness wounds were created at day 0 in a swine model. This study complied with the Animal Welfare Act and followed recommendations in the Guide for the Care and Use of Laboratory Animals. In vitro derived-slough was applied to the wounds at day 0 and NPWTi using VCC was initiated using a 10 min soak period and 3.5 h negative pressure period at -125mmHg. Dressings were changed every 2 or 3 days.

**Results:** Peak maximum principal strain imparted to tissue at -125mmHg with was 27.8% for VCC and 0.8% for VC. The frictional work around the holes in the VCC was 0.18 mJ. When the modelling holes were removed (VC), frictional work across the foam was negligible. The strain energy imparted to tissue with VCC was approximately 0.83 mJ for and 1.36 x 10^-4 for VC. At study term, the granulation
tissue thickness for wounds using VCC was significantly thicker than for VC (9.7 ± 0.1 mm vs 7.4 ± 0.5 mm; p = 0.02).

**Conclusions:** The through holes in VCC leads to higher forces imparted to the tissue than for VC. Frictional work around the 10mm holes which may pin the tissue in place under the holes leading to higher strain energy as the tissue is pulled up into the holes. This work done by the dressing with NPWTi may allow for fracturing of the devitalized tissue which then may be removed during the instillation cycle. The preclinical results corresponded to the FEA. There was more slough removed at earlier timepoints with VCC vs VC with an associated increase in granulation tissue.


---

**P27 | Role Of Hyaluronan Topography On Epithelial To Mesenchymal Transition Of Keratinocytes**

Katherine M. Ballrad, Lichong Xu, Tugba Ozmendir

1 Mercruy Medical Center, The Pennsylvania State University, Rapid City, SD; 2 Nanoscience and Biomedical Engineering, South Dakota School of Mines and Technology, Rapid City, SD

**Introduction:** Epithelial-to-mesenchymal transition (EMT) is a well studied biological phenomenon that takes place during various biological processes including wound healing, Type II EMT. Embryos undergo scarless wound healing while adult wound healing creates a scar. The wound healing extracellular matrix (ECM) varies between adults and embryos. Embryos have higher levels of hyaluronan (HA) and type III collagen fibers, while the adult ECM is marked by denser, aligned collagen bundles and reduced HA. Various studies have reported that HA helps keratinoctye proliferation and migration during wound healing. It remains unknown how different HA topographies affect the proliferation and migration of keratinocytes. To address this gap, we created synthetic matrix analogs that are capable of representing different HA topographies using hyaluronan binding peptide (HABP) studied the role of HA topography on EMT.

**Methods:** Synthesis and Surface Modification of PCL Fibers. Polycapro-lactone (PCL) fibers with diameters 0.5μm and 5μm were made through electrospinning and functionalized with HABP through amylolysis as previously reported [4]. Fiber characterization was performed through SEM and water contact angle (CA) analysis. Cell Proliferation and Migration. Primary human epidermal keratinocytes were used to study EMT. Cell proliferation was studied using Ki67, pico green, and presto blue assays. Cell migration was studied using a transwell migration assay. EMT Marker Quantification. The concentration of common EMT markers was found using western blot. Cell Morphology. Cell morphology was analyzed through fluorescent microscopy where the nucleus and f-actin were stained. In the epithelial state, cells display cortical actin in the membrane cortex, which transitions into stress fibers in the mesenchymal state.

**Results:** We found PCL scaffolds had an average CA of 82.84 ± 17.48° and with the addition of HA the average CA was 77.57 ± 16.52°. With just the addition of HABP to the scaffolds the average CA was 11.88 ± 19.35° and with the addition of HABP and HA the average CA was 4.38 ± 10.72°. The addition of the peptides makes the surface hydrophilic with HABP making the surface completely hydrophilic. Ongoing experiments are currently focusing on understanding the role that HA topography plays on EMT by measuring the concentration EMT markers. Fluorescent images of epithelial cells on the different surfaces will be taken to determine if the addition of a peptide causes cells to take on more of a mesenchymal shape. The results of this experiment will shed light on how topography influences the factors that trigger cells to undergo EMT. The goal of this study was to determine the effect of different the impact of various hyaluronan topographies on cells during Type II EMT.

---

**P28 | Transcriptomic Differences Between Oral And Skin Keratinocytes**

Chen Han, Trevor R. Leonardo, Heidi Yuan, Lin Chen, Luisa A. DiPietro

Center for Wound Healing and Tissue Regeneration, University of Illinois College of Medicine at Chicago, Chicago, IL

**Background:** In comparison to skin wounds, oral mucosal wounds heal more rapidly with significantly less inflammation, faster re-epithelialization, and minimal scarring. Our lab has previously shown that skin and oral excisional biopsy mucosal wounds exhibit site-specific differences in their genetic response to injury and that intrinsic keratinocyte characteristics may be one differentiating factor. The aim of this study was to investigate whether the intrinsic differences between oral and skin keratinocytes would be reflected in their transcriptome at baseline and after injury.

**Methods & Materials:** In this study, we used two keratinocyte cell lines: 1) hTERT-immortalized gingival keratinocytes (TIGK) and 2) spontaneously immortalized skin keratinocytes (HaCaT). RNA was isolated from HaCaT and TIGK at 0-, 6-, and 24-hours post-scratch (N=3). Following DNase treatment, RNA-sequencing was performed and transcriptomic changes in response to injury were compared between HaCaT and TIGK. Genes that were significantly downregulated (p<0.05) in HaCaT versus TIGK underwent gene ontology (GO) enrichment analysis and reactome pathway analysis. GO terms were annotated to biological processes (BP), cellular components (CC), and molecular function (MF). Additionally, HaCaT were stably transfected to overexpress Basic Leucine Zipper ATF-Like Transcription Factor 3 (BATF3-OvExp) or with an empty vector control (EV-Ctrl). Cellular migration and proliferation were assessed for BATF3-OvExp and EV-Ctrl (N=8-10). Expression of genes down-stream of BATF3 were assessed via RT-PCR (N=3-4). HaCaT migration was also assessed following pre- and post-scratch treatment with Interferon (IFN) Type I (N=10-12).

**Results:** Analysis of differentially expressed transcription factors found that BATF3 was significantly downregulated in HaCaT versus TIGK for all time points (p<0.05). BATF3 overexpression significantly enhanced HaCaT migration and expression of down-stream genes...
The Impact of Topical Oxygen Therapy on Wound Healing: Assessing Efficacy and the Influence of Patient Characteristics in a Single-Institution Retrospective Chart Review

Anya Wang1, Martina Brozynski1, Benjamin Jacobs2, Nargiz Seyidova1, Olachi Oleru1, Harvey Himel1
1Department of Plastic Surgery, Icahn School of Medicine at Mount Sinai, New York, NY; 2University of Chicago, Chicago, IL

Background: Aging can diminish wound healing due to insufficient tissue oxygenation. Hyperbaric oxygen therapy (HBOT) poses systemic risks, while topical oxygen treatments (TOT) offer a safer localized alternative. This study examines TOT’s impact on wound healing and how patient and wound characteristics influence its effectiveness.

Methods: A retrospective chart review (8/1/2011 - 7/1/2023) analyzed patients aged 23 to 97 with any wound etiology who used TOT (GWR Medical inc.) after unsuccessful alternate treatments. The device was used 90 minutes daily for four days, followed by a three-day break. Patient demographics (gender, age, race, smoking status, comorbidities, radiation usage, nutrition level), wound details (dimensions, age, location, etiology), and device usage interruptions were collected from EPIC. Comorbidities were subdivided into body-system categories. Wound dimensions were measured using a centimeter ruler. Healing was gauged by percent changes in surface area, depth, and volume between the initial and final measurements. Analysis included two linear mixed effects (LME) models—one for comorbidity groups and one for individual comorbidity counts. Variables with high multicollinearity and variance inflation factor (VIF) > 10 were removed.

Results: From 45 patients, 84 wounds were reviewed, averaging 1.86 wounds and 7.27 comorbidities per individual. Wound sizes ranged from 0.08 cm² to 482.5 cm² (median: 10.25 cm²). About 68% of wounds shrank, 2% remained unchanged, and 30% enlarged. Complete healing was more prevalent in nourished patients (94%), those without radiation history (88%), and non/former smokers (53%, 41%). No wound exceeding 27 cm² completely healed.

The LME model with comorbidity count showed that being male (β = 1.486, p = 0.019), age (β = 1.002, p = 0.032), and bone disease (β = 2.327, p = 0.008) increased surface area healing, while initial depth remained detrimental (β = -0.519, p = 0.007). Age was a non-factor in both models.

Conclusion: Uninterrupted TOT usage promoted surface area, depth, and volume healing, while greater initial depth hindered healing. Given that certain wound and patient characteristics can influence healing with TOT, physicians should consider these factors when tailoring patient treatment plans.
Finite element analysis (FEA) of novel dressing under -125 mmHg produced peak and lower tissue strains of 18% and 4%, respectively, that extended several mm into the wound bed, while ROCF exhibited peak strains of 40% at shallower depths. ROCF also produced downward tissue displacement at wound-foam strut contacts. Downward displacement was seen in the peel and place dressing along the wound edge. Overall, tensile strains were predicted to be more homogenous at the deep wound bed in the peel and place dressing model.

**Conclusion:** Cells respond to imposed forces/strains by producing biochemical stimuli. The homogenous tissue strains and deep propagation of tensile strains seen in the novel dressing FEA model could explain the greater levels of cytokines/chemokines and growth factors compared to ROCF.

1 Novel Peel and Place Dressing (3M Company, San Antonio, TX); 3M™ V.A.C.® Therapy; ™ V.A.C.® Granufoam™


---

**P31 | Comparative Analysis Between 16S rRNA NGS vs Conventional Culture associated with the Treatment Outcome of Diabetic Foot Ulcers**

Sik Namgoong

*Plastic Surgery, Korea University, Seoul, Korea (the Republic of)*

**Background:** Effective treatment of wound-site infections in diabetic foot ulcer (DFU) patients is crucial for a good prognosis. Recently, 16S rRNA next-generation sequencing (NGS) has been the main focus of research for accurately detecting wound-site microbes, which is vital in optimal antibiotic treatment. We compared the conventional culture-based detection method to the 16S rRNA NGS method to predict the DFU treatment outcomes.

**Methods:** Wound-site samples from 47 DFU patients who were treated at Korea University Guro Hospital from February 2021 to November 2021 were analyzed with both conventional culture and NGS methods. We set the primary outcome as the healing status of the DFU. We compared the conventional culture-based detection method to the 16S rRNA NGS method to predict the DFU treatment outcomes.

**Results:** The NGS method detected a broader range of microbial species (Shannon index=1.369 ± 0.755, Simpson index=2.987 ± 1.383) compared to the conventional culture method (Shannon index=0.693, Simpson index=1.269). Sixteen species were found using the two methods, which were all anaerobes. The most significant discordance of detected species was found in the SIBNAD≥3 group (40.79%), and within that group, the patients with an absence of ischemia but poor infection control had the largest discordance (85.22%). Among the microbes detected significantly different between the two methods, *B. fragilis, S. agalactiae, S. aureus, and S. constellatus* were associated with poor prognosis, which were mainly detected in NGS than culture.

**Conclusion:** Early studies now suggest that 16S rRNA NGS may be an effective diagnostic tool for treating diabetic foot infection. We look forward to larger pivotal studies to confirm these initially promising findings.

---

**P32 | Matrix Mechanics Dictate Odontoblast Responsiveness to Photobiomodulation Treatments**

Anya I. Wansha, Mahmud Amin, Adhav Narayan, Victoria R. Oliveira, Praveen R. Arany

*Oral Biology, University at Buffalo, Buffalo, NY*

**Objectives:** Regenerative dentistry promotes the directed differentiation of stem cells. While the predominant focus of these efforts has been genetic manipulations, the critical epigenetic role of extracellular matrix (ECM) mechanics has been poorly investigated. This project aims to dissect the cellular responses of odontoblasts to matrix mechanics by replicating a wound-like environment.

**Methods:** Polydimethylsiloxane (SYLGARD 184) matrices, composed of a 10:1 ratio (base: curing agent) were poured into 12-well polystyrene plates. The polydimethylsiloxane (PDMS) plates were degassed for 5 cycles and cured for 48 hours in a 95° C oven. Mechanical stiffness was then assessed using a Shore A Durometer (Insize). Following sterilization with 70% ethanol, UV treatments, and serum coating, odontoblasts (MDPC-23) were seeded (100,000 cells/well) in hypoxic (1μg/mL CoCl₂ + DMEM 10% FBS) and serum-deficient (0.2% FBS DMEM) to simulate a wound-like environment. Alamar blue assay was performed at 24 hours to assess cell viability, and fluorescence was assessed with a Spectrophotometer (i3Max, Molecular Devices).

An adhesion assay was performed to observe cell-substrate interaction 5 hours after cell seeding. Photobiomodulation (PBM) treatments were performed with a near-infrared (810 nm) laser at 10 mW/cm², 5 min, 3 J/cm², 4.5 p.J/cm² or 1 Einstein. Signaling pathways for TGF-β1 and FAK were investigated using small molecule inhibitors and recombinant protein.

**Results:** The stiffness assessment noted the ratio 10:1 had a stiffness of 1.14 ± 1.65 MPa, 2:1 of 1.16 ± 0.48 MPa, and 40:1 of 0.57 ± 0.27 MPa. These stiffnesses were significantly (n = 3, p < 0.05) lower compared to the polystyrene culture dish control of 5.41 ± 0.96 MPa. No differences were observed in cell adhesion assays in any of the conditions. Statistically significant (n = 3, p < 0.05) changes were observed in cell proliferation with varying matrix stiffness. TGF-β1 and FAK1 signaling was noted to modulate these responses. Ongoing studies are examining the role of PBM activation of these discrete pathways in mediating odontoblast adhesion and survival. Further, direct activation of these molecular pathways is being assessed with Western Blotting for pSmad2/3, pFAK, pAKT, and pPI3K.

**Conclusions:** The results from this study suggest that precision engineering of biomaterial mechanical properties and PBM treatments can...
synergistically promote directed differentiation for optimal dentin regeneration in a simulated wound environment. These findings will next be extended to in vivo animal models for ultimate human clinical translation as a novel Endodontic Regenerative Therapy.

P33  |  The Effects Of N-Acetyl-Cysteine, Gentamicin, and Ciprofloxacin On Biofilm In Vitro In The Presence Or Absence Of Oxidative Stress

Samyuktha S. Vedula, Shayla H. Nguyen, Terrence Lin, Manuela M. Martins-Green
Molecular, Cell and Systems Biology, University of California, Riverside, Riverside, CA

Chronic wounds become colonized by pathogens, primarily bacteria, and these infections often result in the development of biofilms. Unfortunately, treatment with antibiotics alone often fails to resolve chronic wound infections, in part because antibiotics are ineffective at killing bacteria in biofilms. Persistent biofilms in chronic wounds significantly delay wound healing. The purpose of this study was to determine the effects of N-acetyl-cysteine (NAC), Ciprofloxacin (Cipro), and Gentamicin (Gent) on Enterobacter cloacae (Ec), Pseudomonas aeruginosa (Pa), and Staphylococcus xylosus (Sx) biofilm, alone and in combination. We also tested the effects of these treatments in presence or absence of oxidative stress. 1x10⁶ cfu/200μl of bacteria were cultured in 96-well plates and allowed to form biofilm for 24 hours. The biofilms were then treated with NAC (20 mg/ml) or NAC + Gent (100 μg/ml) + Cipro (10μg/50μl) for another 24 hours. The biofilm was stained with 0.1% crystal violet, distained with 95% ethanol, and analyzed at OD590 to measure the amount of biofilm present after treatment. To create the conditions for oxidative stress, H 2 O 2 at a final concentration of 500μM was used. NAC had a significant effect on Ec, Pa, and Sx individually in decreasing the amount of biofilm, but its effects were significantly lessened when used under oxidative stress conditions. For Ec, Pa, and Sx individually, NAC + Gent + Cipro was less effective than NAC alone at decreasing the amount of biofilm in non-oxidative stress conditions. There was no significant difference for Ec and Sx alone between NAC and NAC + Gent + Cipro under oxidative stress conditions. However, for Pa, NAC + Gent + Cipro was more effective at decreasing the amount of biofilm under oxidative stress conditions. For the combinations Ec+Pa, Ec+Sx, and Pa+Sx, there was no significant difference between the effects of NAC alone versus NAC + Gent + Cipro under conditions without oxidative stress. Under conditions with oxidative stress, NAC + Gent + Cipro was significantly better at decreasing the amount of biofilm for the combinations Ec+Pa, Ec+Sx, and Pa+Sx. For the combination Ec+Pa+Sx, without conditions of oxidative stress, there was a significant difference between NAC alone and NAC + Gent + Cipro, as the latter was more successful at decreasing the amount of biofilm. Under conditions with oxidative stress, there was no significant difference between NAC alone and NAC + Gent + Cipro for Ec+Pa+Sx. In conclusion, NAC, has proven to be able to significantly decrease the amount of biofilm with and without oxidative stress under most conditions. However, under other conditions NAC is aided by Gent and Cipro. Regardless of whether the bacterial strains are alone or in combination with each other, the effect of NAC is significant in decreasing the amount of biofilm, though sometimes it needs the assistance of the antibiotics.

P34  |  Novel Anti-Biofilm Treatment For Hidradenitis Suppurativa Tunneling Wounds Modulates Host-Pathogen Response To Promote Scarless Wound Healing

Nathan Balukoff², Tammy Gonzalez², Divya Chopra², Nicole Vecin², Caralin Schneider², Marjana Tomic-Canic², Matthew Myntti¹, Hadar Lev-Tov², Irena Pastar²
¹Next Science Ltd, Jacksonville, FL; ²Department of Dermatology and Cutaneous Surgery, University of Miami, Miami, FL

The function of the epidermis and response to wounding are significantly compromised during the development of the cutaneous inflammatory condition hidradenitis suppurativa (HS). Resident HS keratinocytes erroneously migrate into the surrounding area forming pathognomonic epithelial, intra-dermal tunneling wounds, which are primary drivers of chronic inflammation. In addition, HS tunneling wounds are colonized by anerobic bacteria that form treatment-resistant biofilms. Persistent biofilms are hypothesized to contribute to an aberrant inflammatory response and, subsequently, the relapsing nature of HS tunnels. Here we performed the first pilot longitudinal study to evaluate the effectiveness of antibiofilm therapy for HS tunneling wounds.

HS Subjects (n=15) with confirmed tunnels were recruited to the study and pre-treatment lesional tissue was collected to evaluate the host response and microbiome composition. Subjects then instilled the antibiofilm surfactant gel daily into the tunneling wounds, and tissue was collected 28 days after the procedure for evaluation of the inflammatory response and bacterial load by 16s rDNA sequencing. RNA was isolated before and after treatment and host response was analyzed with Nanostring’s Ncounter technology.

We observed 93% clinical, tunneling wound resolution and overall reduction of scarring at an average of 12 days after daily instillation of antibiofilm surfactant gel in HS tunneling wounds. Clinical findings correlated with the significant attenuation of the inflammatory response after anti-biofilm treatment, with marked reduction in INFg, IL-17, IL-6, IL-8, and TNFa signaling. Furthermore, the treatment resulted in reduction of bacterial load, and restoration of microbial dysbiosis reflected in lower abundance of anaerobic pathogens and restoration of commensal bacterial genera.

Our study highlights the importance of therapeutic targeting of bacterial biofilms in HS tunneling wounds. We conclude that anti-biofilm therapeutics offer a novel treatment approach to suppress inflammation, restore healthy microbiome and alleviate clinical symptoms in affected patients.
P35 | Exploring MicroRNA-Mediated Pathways In Wound Healing In Patients With Chronic Venous Leg Ulcers

Magali Rezende de Carvalho, Debra E. Lyon
University of Florida, Gainesville, FL

Purpose of the Study: The incidence of Chronic Venous Leg Ulcers (CVLU) is escalating among the adult and elderly population, posing a substantial burden on patients and the healthcare system due to its high recurrence rate and chronicity. This literature review aims to explore the influence of microRNAs associated with wound healing in CVLU patients.

Methods: A comprehensive search on PubMed and EMBASE was conducted in October 2023 to identify studies analyzing microRNAs involved in CVLU wound healing. Ninety-six records published in English over the past decade were retrieved, leading to the selection of 13 studies for full-text review. After applying inclusion/exclusion criteria, five studies were chosen for detailed analysis.

Results: Biopsy samples from 56 CVLU patients and one sample from adipose tissue were collected, alongside three studies utilizing wound samples from healthy volunteers for comparison. Overexpression of miR-221, miR-222, miR-92a, and miR-301a-3p was found to negatively impact angiogenesis. Conversely, miR-296 overexpression upregulated VEGFR2 expression, facilitating angiogenesis. miR-301a-3p upregulation exacerbated vascular endothelial cell damage by targeting IGF1, mediating inflammatory responses, increasing HUVEC apoptosis, and elevating oxidative stress, ultimately impairing wound healing. Additionally, miR-19a/b and miR-20 upregulation were associated with downregulation of keratinocytes' inflammatory response through the NF-kB signaling pathway, restoring wound healing in vitro and in vivo using an animal model. Conversely, miR-34a and miR-34c upregulation impaired wound healing by enhancing keratinocytes' inflammatory response through the miR-34-LGR4 axis.

Conclusion: MicroRNAs play a pivotal role in regulating angiogenesis, inflammatory responses, cell migration, and wound healing. Identifying specific microRNAs and their target pathways offers valuable insights, guiding researchers towards potential therapeutic targets for treating chronic venous leg ulcers.

P36 | Differences In Subcutaneous Thickness and Location Affecting Wound Healing in the Swine Model

Guillermo Villa-Martinez1, Hsin-ya Yang1, Anthony Gallegos1, Sriansh Pasumarthi1, Amy Lesneski2, Kirstie Shulman2, Victoria Hammitt3, Linda Talken3, Elham Askalooni2, William Ferrier3, Rivkah Isseroff4, Marco Rolandi2

1Dermatology, UC Davis, Sacramento, CA; 2Engineering, UC Santa Cruz, Santa Cruz, CA; 3Stem Cell Research, UC Davis, Davis, CA

The use of the domesticated swine proves to be an integral part of advancing our understanding of dermatology in human skin due to its similarities to humans and the use of it as a model. A few reports in the literature have noted differences in healing in excisional wounds in different cephalo-caudal anatomical locations. We hypothesized that the differences in healing may be related to possible differences in the thickness of the dermal or adipose layers, as they contribute to the healing process. Here we addressed this question of how these layers vary in thickness when excisional wounds are created in different anatomical locations, albeit when excising down to the same anatomical landmark of the panniculosus carnosus muscle in the attempt to create equivalent wounds. Eight full-thickness wounds (1.6cm in diameter for 2cm2 area) were created along the dorsal region of the Yorkshire/Landrace crossbreed pigs along a cephalo-caudal axis, with each wound being 3cm separated from the adjacent one. Both the depth of the resultant wound and the excised tissues were measured. At day 7 post-wounding, the entire wound was excised and fixed and sectioned, and the thickness of the dermal and subdermal tissues at the wound margin was measured histomorphometrically. Wound depths of the excised cranial wounds averaged 8.8cm while those in the caudal area averaged 6.7cm (N=28 wounds, 0.01 P-value). Histomorphometric analysis of the dermal and subdermal layers adjacent to the excised wounds showed that in the cranially located wounds the dermis averaged 2515um as compared to 2747um in caudally located wounds (N=24, 0.05 P-value), and the subcutaneous layers measured 6674um and 4584 in the cranial and caudal wounds respectively (N=24, 0.0001 P-value). This study demonstrates that despite excising down to the same anatomical landmark in an attempt to create identical wounds, the dermal and subcutaneous tissues are thicker in cranial areas as compared to the caudal wounds. Surprisingly, however, on day 7 post wounding, there is no difference in the wound re-epithelization between these two areas. Additional studies examining healing at later time points may reveal differences in healing.

P37 | Novel Therapy For Long-Term Pressure Injury Prevention: Preliminary Evaluation Of A Biomimetic Implanted Stimulator For Automated Regular Weight Shifting

Kath M. Bogie3, Douglas Shire3, Joseph Lerchbacker3, Christian Zorman2

1Louis Stokes Cleveland VA Medical Center, Cleveland, OH; 2Case Western Reserve University, Cleveland, OH

Background: Pressure injury (PrI) prevention is a major challenge for many people with limited mobility, often leading to prolonged bedrest, hospitalization and even death. Clinical practice guidelines recommend weight-shifting every 20 minutes, but this is difficult when busy with activities of daily living. Prevention thus remains a major challenge which we have found that intermittent gluteal neuromuscular electrical stimulation (iGSTIM) can address. Unfortunately, while surface gluteal electrical stimulation has been used for short periods it has limited efficacy for long term use. A fully implanted iGSTIM system was requested by many people desperate for a new alternative to PrI prevention. iGSTIM using bilaterally implanted electrodes provides...
sustained and effective, regular and automatic weight-shifting, which increases muscle bulk, reduces intramuscular adipose tissue and maintains improved tissue health over many years.

Methods: Newly emerged technologies have enabled us to build soft flexible stimulators and electrode arrays that are implantable and biocompatible for implantation. The mechanically biomimetic and functionally flexible 4-channel stimulator, flexSTIM can provide clinically relevant weight-shifting for up to 14 hours/day. Preliminary biocompatibility testing of flexSTIM has been completed in five New Zealand White rabbits.

Results: Biocompatibility testing of system components implanted for a six month period found that flexSTIM and the intramuscular electrodes were well tolerated. We also have successfully created and reproduced a rabbit spinal injury survival model with animals exhibiting sustained loss of unilateral hind limb function while remaining healthy with independent bowel and bladder function.

Conclusions: Further development will include review and refinement of pre-clinical protocols as needed for evaluation of sustained flexSTIM function, reliability and safety.

P38 | Detection Of Pseudomonas Aeruginosa Concentration Via Synthetic Biosensor For Quorum Sensing Autoinducers

Skylar A. Leslie, David Karig, Jordon Gilmore
Bioengineering, Clemson University, Central, SC

Purpose: The purpose of this study was to develop a real-time chronic wound infection monitoring sensor through the evaluation of a whole-cell Escherichia coli biosensor for the detection of N-(3-Oxydodecanoyl)-L-homoserine lactone (3OC12HSL), an important autoinducer in the quorum sensing network of Pseudomonas aeruginosa.

Methods: To simulate chronic wound fluid conditions in vitro, various concentrations of Lysogeny broth (LB) were combined with artificial wound fluid exudate (AWFE, Biochemzaone) to grow P. aeruginosa. This fluid was then used to determine ranges of 3OC12HSL detectable by our synthetic biosensor. P. aeruginosa was grown in varying concentrations of LB and AWFE, starting at 25% LB mixed with 75% AWFE and moving down to 5% LB and 95% AWFE; negative controls of (100% LB) and positive controls (100% AWFE) were also tested. A growth curve for the bacteria was generated via absorbance detection at 600nm. A crystal violet assay was also performed at 590nm for all media types.

A synthetic gene network, including the lasR, amiICP (colorimetric reporter with blue color), and kanamycin resistance genes, were added to a DH5α E. coli host and selected by plating in LB agar + 50μM/mL kanamycin. The colonies were picked and grown overnight in LB with kanamycin. Cultures are then resuspended in fresh LB with kanamycin and added to 12 conical tubes. 10μM/mL of exogenous 3OC12HSL was added to the first tube and serially diluted by 1mL through the final tube (1:4 dilution each tube). The different concentrations and bacterial and media controls were plated in triplicate in a 48-well plate and read at 588nm, 600nm, and 700nm to quantify amiICP expression.

Results: Ten tests were performed for the differing media concentration growth curves in four replicates (n = 40) per concentration (total n = 280). A single-factor ANOVA and Tukey tests were used to determine which concentrations differed for biofilm analysis. There was a statistically significant increase in biofilm growth for the 100% LB condition compared to any other condition containing AWFE where p = 0.05. Additionally, biofilm growth decreased with increasing concentration of AWFE across all conditions where p = 0.05. Two tests were performed to detect exogenous 3OC12HSL in three replicates (n = 6). Currently, our designed biosensor has been able to detect concentrations of 3OC12HSL above 0.15nM/mL of 3OC12HSL.

Conclusions: The concentration of LB plays a significant role in P. aeruginosa growth and biofilm development. With future tests, we can predict which concentration accurately describes clinical samples. The synthetic biosensor can detect exogenous 3OC12HSL to about 0.15nM/mL in liquid cultures. Further testing will allow for more accurate quantification of 3OC12HSL toward real-time infection diagnosis.

P39 | Effectiveness And Safety Of Negative Pressure Wound Therapy On Melanoma Surgical Wounds

Kyoung Ae Nam
Dermatology, Severance Hospital, Seoul, Korea (the Republic of)

Background: Negative pressure wound therapy (NPWT), a wound dressing system that provides sub-atmospheric pressure throughout the wound site, promotes wound healing, and reduces surgical complications. Although it is contraindicated in malignant wound due to the potential risk of tumorigenesis, the evidence is limited. To compare tumor recurrence and wound healing performance, and surgical complications to provide evidence for the use of NPWT on melanoma-resected wounds.

Methods: We retrospectively reviewed the medical record of 232 patients who were histopathologically diagnosed with acral lentiginous melanoma without nodal and distant metastasis between Jan 2006-Feb 2020. One hundred and seventy nine patients received NPWT, and 53 patients received conventional surgical dressing.

Results: Fifty one (28.5%) patients in the NPWT group had recurrence of which 18 (10.1%) were local recurrence, 17 (32.1%) patients who received conventional surgical dressing had recurrence of which 5 (9.4%) was local recurrence. There were no significant differences in recurrence free survival between both group (Log rank test, P = 0.701).

Patients who received NPWT with skin grafting showed significantly faster wound healing compared to those who received conventional surgical dressing alone, and NPWT without skin grafting (P = 0.001).

Patients who received NPWT had lower surgical site infection rate than conventional surgical dressing (15.1% vs 28.3%, P = 0.028).

Conclusion: NPWT does not significantly increase tumor recurrence in melanoma-resected wounds. Compared to conventional surgical
dressing, NPWT offers several advantages in promoting wound healing and reducing surgical site infection.

**ABSTRACTS**

**P40 | Copper Nanoparticle-Based Multifunctional Scaffolds For Wound Healing**

Alhussain A. Ojaym, Davis Burleson, Songping Huang, Min-Ho Kim
Kent State University, Kent, OH

The pathophysiology of non-healing wounds has been associated with the colonization of multispecies bacteria as well as poor vascularization in wounds. Thus far, each of the above aspects have been separately investigated and an integrated approach to simultaneously addressing these issues in a cost-effective single drug delivery platform has yet to emerge. The objective of this study was to develop copper nanoparticle (CuNPs)-based injectable scaffolds with antibacterial as well as proangiogenic properties for wound healing. Our strategy relies on the current scientific knowledge that (1) copper has great potential to as antimicrobial agent for both topical and systemic administration, while it is less harmful to the host cell because copper is an essential metal for life; (2) copper is a co-factor for many angiogenic promoters and mediators, and can switch on such molecules from the quiescent to pro-angiogenic state; (3) Ascorbic acid exhibits dual functions of pro-oxidative and antioxidant activities depending on its concentrations. In this study, CuNPs (20-120 nm) were synthesized using a green hydrothermal synthesis method. The antibacterial and proangiogenic capabilities of CuNPs were fine-tuned by optimizing the dose of vitamin C (VC) that reacts with CuNP. The antibacterial activity of CuNPs was significantly enhanced when combined with higher concentrations of VC, which synergistically enhanced a Fenton reaction for reactive oxygen species generation (ROS). The threshold level of VC for triggering a Fenton reaction with CuNP was measured to be 10 mM, where the MICs of CuNPs were measured to be 20 mM for both (methicillin sensitive and methicillin-resistant *S. aureus*), and 5 mM for both (drug-sensitive and drug-resistant *P. aeruginosa*), suggesting a potent antibacterial activity of CuNP/VC cocktail against broad-spectrum bacterial species. The proangiogenic activity of CuNPs was assessed by tube formation assay, in which the treatment of CuNPs (80 nm) on HUVEC significantly increased endothelial tube formation in a dose dependent manner at lower doses of VC (<100 mM). An injectable formulation of hydrogel scaffold was prepared by incorporating CuNP and VC (at 10xMIC) in a thermoreversible Pluronic F-127 hydrogel (25%), which enabled controlled release of CuNPs and VC. The bactericidal capacities of the CuNP/VC-loaded hydrogel formulation was assessed using a standard well diffusion assay against *S. aureus* and *P. aeruginosa*. The results showed a significantly increased inhibition of bacterial growth for the gel with both CuNPs and VC, compared to the gel with either CuNP or VC only (p<0.05). In summary, our results support the feasibility of CuNP-based multifunctional hydrogel scaffold that facilitates the eradication of bacterial pathogens as well as proangiogenic response for wound healing.

**P41 | Gradient Skin Barrier Response Caused By Acute Injury In A Clinical Skin Stripping Model - Implications For Within-Subject Study Design**

Madeline Hakala1, Tim House2, Tage Carlson1, Abram D. Janis1
1Hollister Incorporated, Libertyville, IL; 2Dermico LLC, Broomall, PA

**BACKGROUND.** Transepidermal water loss (TEWL) is an indicator of skin barrier disruption following skin stripping injury. Consequently, TEWL is routinely used to assess skin response to candidate ostomy barrier formulations, with low TEWL readings suggesting that a barrier formulation is gentle on the skin when removed. When utilizing TEWL, however, studies have failed to account for potential differences, or interactions between anatomical sites on the abdomen. This study sought to determine whether TEWL response to repeated mechanical stripping was equivalent at different distances from the abdominal midline & across bilaterally symmetrical sites on the abdomen of healthy volunteers.

**METHODS.** Following acclimation in an environmentally-controlled room & measurement of baseline TEWL at each site, a coupon of an aggressive hydrocolloid adhesive A was applied to site 1 (S1) & 3 coupons of a gentle hydrocolloid adhesive B were applied to a row of 3 sites (S2, S4, S5, right to left) across the abdomen of 50 healthy subjects. A midline untreated site (S3) served as a control. This arrangement of sites permitted comparison of bilaterally symmetrical treatments to adjacent treatments to detect anatomical effects on TEWL in response to injury. Seven cycles of adhesive application & removal after 45min were performed. TEWL readings were measured at the 5 sites after the 6th & 7th removals.

**RESULTS.** After the 7th removal, the mean TEWL value for S1 was significantly higher (p< 0.05) than the other 4 sites, which was expected as this site was exposed to aggressive adhesive. The mean TEWL values for S4 & S5 on the opposite side of the abdomen were not statistically different, demonstrating that distance from vertical midline did not have an impact on TEWL when the formulations were equivalent. Interestingly, the mean TEWL value at S2 (gentler adhesive) was significantly higher than the bilaterally symmetrical S4 site (p< 0.05), despite being the same formulation & their equal distances from the midline. Distance from the site treated with the aggressive formulation A at S1 was the only identified difference between S2 & S4, suggesting that proximity to the injury caused by the aggressive formulation led to a gradient of greater TEWL in the adjacent sites treated with the gentler formulation.

**CONCLUSIONS.** Addressing the study's hypothesis of bilaterally symmetrical injury responses was not possible, due to the finding that proximity to the injury caused by an aggressive barrier formulation influenced nearby TEWL readings, revealing that acute skin stripping injuries cause skin barrier disruption that extends beyond the point of injury. Further research is needed identify the cause of this gradient effect & the implication of this effect on study designs comparing adhesives within an individual subject.
ABSTRACTS

P42  |  SWAZA-2: Hydrogel Oxygenation Therapy For Treating Burn Wounds

Nicole Jones, Ankur Samantha, Niki Santo, Jayakumar Rajadas, Artem A. Trotsyuk
Swaza, Inc., Mountain View, CA

BACKGROUND: Burns have important functional and psychosocial implications for patients. Decades of wound healing research have demonstrated a critical window within the first 24 hours after wounding during which there is a “switch” from scarless wound healing to scarring. Recently, cell-based therapies have been proposed as an option for improving healing and reducing scar formation in burn wounds. Yet challenges remain for scale up and broad bioavailability of the therapy. Oxygen chambers have long been used in supportive wound care and oxygenation therapy presents an improved way reduce scarring and accelerate wound closure.

METHODS: We have developed a novel hydrogel that recapitulates results seen in a hyperbaric oxygen chamber, without the need for a chamber in Swaza-2. This hydrogel, combined with Swaza-1, a perfluorocarbon-based oxygen nanoparticle, was tested on a murine contact burn model in C57BL/6 mice to measure the regenerative capabilities of Swaza-1-infused hydrogel and its effects on scarless wound healing.

RESULTS: Wounds treated with Swaza-2 demonstrated accelerated healing and time to re-epithelialization, brought about by increased VEGF and SDF1 expression and significantly higher neovascularization and collagen deposition (p<0.05). We also observed an increase in the expression of pro-angiogenic genes MCP-1, VEGF, and SDF-1 at both the protein and mRNA level (p<0.05). Expression of pro-fibrotic and pro-inflammatory genes was downregulated. On average, Swaza-2 wounds closed 4 days earlier when compared to controls. Furthermore, Swaza-2 treated burns exhibited reduced scar area when compared to the untreated control.

CONCLUSION: We have developed an oxygen-hydrogel therapy for treating burns, with demonstrated pro-angiogenic, fibromodulatory and immunomodulatory effects. We plan to further evaluate the efficacy of Swaza-2 in a large animal model.

P43  |  Factors Affecting Complication Rates For Chest Wall Tumor Excision With Plastic Reconstruction

Namrata V. Chintalapati, Kirtana Sandepudi, Stuti P. Garg, Robert Galiano
Plastic Surgery, Northwestern University, Chicago, IL

Background: The main approach for treatment of chest wall tumors is chest wall resection, which can generally be carried out with minimal risk of complications including dehiscence, seroma, hematoma, and infection. However, there remains a knowledge gap in ascertaining demographic differences between patients who develop complications.

Methods: The All of Us database was queried for patients with excision of a chest wall tumor with plastic reconstruction, with or without mediastinal lymphadenectomy. Dataset v7 was utilized to extract race, gender, income, and comorbidity data along with respective rate of development of dehiscence, seroma, hematoma, or infection within 30 days.

Results: A total of 1,832 patients who underwent excision of a chest wall tumor with plastic reconstruction with or without mediastinal lymphadenectomy were included, of which 18.7% were white, 23.8% were black, 1.0% were Asian, 2.4% were mixed or other, and 54% did not answer. There was no significant difference in proportion of patients developing complications on the basis of race. While patients who self-identified as male made up a smaller proportion of total patients, they were more likely to develop complications (5.1%) than patients who self-identified as female (2.3%) (OR = 2.26, 95% CI 1.35-3.81) There was no significant difference in development of complications between patients with an annual household income of greater than $100,000 compared to patients with an income of less than $100,000 (OR 0.74, CI 0.31-1.75). Patients who developed complications were more likely to have comorbid renal failure (OR 1.82, CI 1.03-3.03) or congestive heart failure (OR 2.65, CI 1.31-5.36)

Conclusion: Patient race and income are not strongly correlated with rates of wound dehiscence, seroma, hematoma, or infection within 30 days of chest wall plastic reconstruction. However, male patients have a higher rate of complications than female patients, and patients who develop complications are more likely to have comorbid renal failure or congestive heart failure. These findings contribute valuable insights into wound healing dynamics. Investigation into these factors could pave the way for novel therapeutic approaches and improved wound management strategies.

P44  |  Skin Blot Examination For Changes In Systemic Cytokine Profiles Induced By Indirect Irradiation Of Ultraviolet-Free Light On Atopic Dermatitis Patients: An Intervetional Pre-Post Study

Mana Miyazaki1, Kazuhiro Ogai2, Yoko Hasegawa1, Hiromi Tobe3, Katsunori Kato4, Nana Nuka2, Yoko Onishi2, Masaru Matsumoto2, Chizuko Konya2, Takeo Minematsu2
1Department of Bio-engineering Nursing, Ishikawa Prefectural Nursing University, Kahoku, Ishikawa, Japan; 2Department of Adult Nursing, Ishikawa Prefectural Nursing University, Kahoku, Ishikawa, Japan; 3Department of Child Health Nursing, Ishikawa Prefectural Nursing University, Kahoku, Ishikawa, Japan; 4Graduate School of Nursing, Ishikawa Prefectural Nursing University, Kahoku, Ishikawa, Japan

Atopic dermatitis (AD) is a skin disease characterized by dry, itchy, and inflamed skin. Ultraviolet (UV)-free light therapy could be considered as a treatment of AD when medication is not effective and UV is considered harmful to the skin. UV-free light therapy is reported to be effective even when the light is irradiated to the normal skin far from the AD-affected sites; however, the mechanisms underlying this indirect effect remain unclear. We investigated the changes that occur
with improvement in AD by indirect irradiation using UV-free light, with a focus on cytokine changes.

This interventional pre-post study enrolled five non-AD individuals (one male and four female, aged 21–40) and four AD patients (one male and three female, aged 21–48) after providing written informed consent. The intervention involved exposing the soles of the feet to UV-free light for 15 min three times weekly for six weeks. The Patient-Oriented Eczema Measure (POEM) was used to assess AD during intervention. We also collected systemic proteins from the inner side of the forearm using a skin blotting method, which can non-invasively capture circulating proteins through the skin, pre- and post-intervention. Cytokine levels in the collected samples were measured using a human cytokine array kit. This study was approved by the medical ethics committee of the university where the study was conducted (approval No. 2023-153-3).

Of the four participants with AD, two showed improved POEM scores (from 19 to 16 and 10 to 5, respectively), whereas the other two showed either no change (3 to 2 in POEM) or deterioration (6 to 12 in POEM). The cytokine profiles in AD revealed 15 upregulated and 19 downregulated cytokines. Among them, T-helper cells regulators (such as macrophage inflammatory protein (MIP)-3α, insulin-like growth factor (IGF)-1, IGF binding protein (IGFBP)-3, and IGFBP-4) and responders (including interleukin (IL)-5, IL-12, and IL-13) underwent changes due to UV-free irradiation.

In summary, our skin blot examination results revealed changes in T cell-related cytokines (MIP-3α, IGF-1, IGFBP-3, IGFBP-4, IL-5, IL-12, and IL-13) following irradiation of unaffected skin with UV-free light in AD patients.

---

**P45 | Bacterial Protease-Responsive Shape Memory Polymers For Infection Surveillance & Treatment Of Chronic Wounds**

Thalma Orado¹, Jessica Peterson², Samantha Zysk¹, Richard Chandradat², Xiaocun Lu³, Mary Beth Monroe¹

¹Biomedical & Chemical Engineering, Syracuse University, Syracuse, NY; ²Chemistry & Biochemistry Department, Texas State University, San Marcos, TX; ³Department of Chemistry & Biomolecular Science, Clarkson University, Potsdam, NY

The purpose of this study was to determine the sensitivity and specificity of a polyurethane shape memory polymer with bacterial protease-sensitive peptides (PUR-PEP) towards bacteria. Additionally, we explore the incorporation of molecular force sensors known as spiropyrans (SP) into the polymer to improve visible surveillance of infected wounds.

Strained PUR-PEP samples (n=3) were incubated in mammalian enzymes (matrix metalloproteinase-1, trypsin, and lysozyme), and in bacterial enzymes (S. aureus V8 and beta-lactamase) at 37°C for 10 days. Sample dimensions were measured using digital calipers every 24hr, and recovery ratios were determined based on differences in length. Strained PUR-PEP samples (n=3) were also incubated in serial dilutions of S. aureus (10^7 to 10^8 colony forming units (CFUs)) in stasis buffer for 7 days at 37°C. Samples were imaged daily with a camera, and dimensions were quantified using ImageJ software. To enable color change with shape change, 2.5 g of polymer was dissolved in chloroform, and 1.25 mg of photochromic SP was added. The mixture was poured into a Teflon dish until solvent evaporated, and the resultant films were dried in a vacuum oven. Samples were cut from the films, heated to 100°C, strained lengthwise, and then cooled. Samples were then imaged on a fluorescence microscope using the green channel or irradiated using a UV lamp (λmax= 365 nm) and imaged with a camera to assess color changes before and after staining.

Strained PUR-PEP samples underwent significant shape recovery (~55%) (p<0.05) in both mammalian and bacterial enzymes. The material also recovered in all tested concentrations of bacteria, and a one-way ANOVA showed no association between bacteria concentration and shape recovery (F (1,5) = 0.68, ns). The strained PUR with SP exhibited increased fluorescence and luminiscence when irradiated with a UV lamp. PUR-PEP recovery in mammalian enzymes indicates that the material chemistry requires tuning to improve its specificity towards bacterial proteases. However, the material recovery in all concentrations indicated high sensitivity and the potential to be utilized in detecting low grade or early infections. The photochromic response visible by fluorescence microscopy was due to increased absorbance by SP upon straining the PUR films. These are promising results as SPs could be used as a sensor to visually indicate changes in the shape of our PUR wound dressings, providing easily detectable color-based surveillance of infection in wounds.

---

**P46 | Hyaluronic Acid Based Adipose Tissue Derived Extracellular Matrix Scaffold (Scaffiller) In Wound Healing: Histological And Immunohistochemical Study**

Young-Joon

Plastic surgery, The Catholic university of Korea, Seoul, Other, Korea (the Republic of)

**Background:** Adipose tissue is considered the most accessible and optimal source of extracellular matrix (ECM) products in clinical settings. In our prior study, we evaluated the effectiveness of human adipose tissue-derived ECM (adECM) sheets as a wound dressing material. To enhance healing potential and cost-effectiveness, we modified adECM sheets by adjusting ECM concentration and incorporating crosslinked hyaluronic acid (HA). Adipose tissue was obtained from healthy donors, processed, and casted into ECM sheets.

**Methods:** Crosslinked HA was added to create ECM-HA sheets (Scaffiller, Medikan, Korea). In vitro analysis involved seeding adipose-derived stem cells (ASCs) onto porous ECM-HA sheets and evaluating cell survival rate and cytokine array after 3 days. In vivo efficacy was assessed by applying ECM-HA sheets to full-thickness wounds in a rat model.
model, with HA-based dressing and adECM sheets as control groups. Re-epithelialization and collagen deposition were examined through histopathological examinations, while immunohistochemistry was used to assess CD31, α smooth muscle actin (α-SMA), and Tenascin C expression as contributing factors to wound healing.

**Results:** The extracted ECM components accounted for approximately 5% of the original tissue volume, with ECM-HA sheet production efficiency being six times higher than adECM sheet. In vitro analysis revealed favorable ASC survival rates and increased angiogenic and bioactive cytokine levels in ECM-HA sheet. Macroscopic evaluation showed enhanced healing rates, while histological analysis demonstrated improved epithelialization, thicker dermis, increased collagen deposition, and enhanced vascularity in the ECM-HA group. Notably, decreased α-SMA expression and increased Tenascin C expression were observed in the ECM-HA group.

**Conclusion:** Our study successfully fabricated ECM-HA sheets incorporating adECM and HA, resulting in improved material stability, cost-effectiveness. ECM-HA sheets exhibited increased growth factor production, improved wound healing rates, collagen deposition, angiogenesis, and reduced myofibroblast activity. ECM-HA sheets hold promise as scaffolds for adipose-derived stem cells, showcasing significant therapeutic potential for wound healing applications.

---

**P47 | The Role of Polyvinyl Alcohol Antibacterial Foam on Debridement in Lower Extremity Chronic Wounds: A Clinical Study**

Eric Lullove
West Boca Center for Wound Healing, Coconut Creek, FL

Use of Polyvinyl alcohol has been widely used in the chronic wound care space for many years. The role and function of the foam has been utilized for exudate management and antimicrobial barriers as both primary and secondary dressings for extracellular matrices and even in cases with NPWT. This study specifically looked at the feasibility of using PVA foam for debridement purposes, in cases where all subjects could not undergo surgical excisional debridement. The study was conducted under an IRB review with the single site investigator. 20 subjects were enrolled for the clinical study. The PVA foam was applied weekly for 4 weeks duration as the primary dressing after saturation with sterile saline and a sterile dry secondary bandage. Surface area wound measurements were conducted using the Molecult light iX device with documentation of reduction of bacterial immunofluorescence via wound photography. Findings showed that after 4 weeks, average wound surface area reduced approximately 54% from initial measurements and bacterial fluorescence was absent by week 4 in all subjects. These findings show that in patients with chronic lower extremity wounds that cannot tolerate surgical sharp debridement, the use of PVA foam can be an adequate adjunct for mechanical debridement modality as well as providing the patient an adequate antimicrobial dressing.

---

**P48 | Autologous Skin Cell Suspension In Complex Wounds: A Case Series And Protocol**

Cheryl Acampora, Lisa Gould
Surgery, South Shore Hospital, Weymouth, MA

**Introduction:** Non-healing wounds fail to proceed through the normal stages of healing in a timely fashion, disproportionately affect the elderly and comorbid, and result in an enormous medico-socio-economic burden. Unfavorable patient demographics and wound characteristics lead to poor response to standard topical wound care. Primary closure is often unattainable, and meshed skin grafts carry elevated risk of poor take. The Autologous Skin Cell Suspension (ASCs) technology, recently FDA-approved (6/7/23) for full-thickness skin defects, offers a solution by minimizing donor size and stimulating epidermal regeneration. Real-world data is crucial for understanding optimal application of ASCs. We present a pilot study to develop an algorithm that will guide the use of ASCs for challenging wounds.

**Methods:** A prospective analysis of patients with complex wounds reconstructed with ASCs by a single surgeon, Aug-Nov 2023, was conducted. Clinical photographs, demographics, treatments, and complications were reviewed. After patient optimization and wound bed debridement, the decision to use ASCs included an assessment of healing potential based on comorbidities and history of poor wound healing. Risk-benefit analysis was conducted, with ASCs favored in patients at elevated risk of infection, delayed healing, bleeding, or high drainage at the donor/treatment sites, and in those with poor pain tolerance or areas difficult to immobilize for graft take. Assessment of cost effectiveness including device cost, total cost of care in terms of clinic follow-up, revisions, and need for additional advanced wound therapies was considered.

**Results:** Six patients (5 male, mean age: 64.8 years) with subacute and chronic wounds underwent reconstruction with ASCs (1 diabetic, 1 diabetic on dialysis, 1 chronic kidney disease, 2 with poor wound healing history). Following serial sharp debridement, either dermal regenerative template, topical wound care, and/or negative pressure wound therapy (NPWT) was utilized to prepare wounds for grafting. ASCs was applied to wounds with healthy granulating beds. Some wounds were treated with thin 3:1 meshed split-thickness skin grafts and oversprayed with ASCs. A non-adherent contact dressing was applied for ≥1 week, combined with compression wraps or NPWT. Three wounds have healed to date with durable epidermis, early repigmentation, and rate of healing faster than expected (mean healing time: 11.5 weeks).

**Discussion:** ASCs can accelerate healing of complex wounds while reducing donor size in the presence of multiple factors compromising healing or poor graft take and represents a viable surgical option for patients with multiple comorbidities. With expanding use of this technology, determining the appropriate use in wound care is critical. This pilot study is the first step toward a larger trial that will guide clinical decision making for grafting complex wounds. Additional cases are in progress.
P49  |  Does ClimateCare® Improve Pressure Ulcer Outcomes After Surgical Closure?

Stuti P. Garg, Anitesh Bajaj, Emmanuelle Hanna, Iris Bai, Diana Griffin, Robert Galiano
Northwestern University, Chicago, IL

Background: Pressure ulcers (PU) are injuries to the skin and underlying tissue that can have significant morbidity with the presence of complications such as dehiscence and necrosis. ClimateCare® is a mattress coverlet system that aims to maintain optimal skin moisture, temperature, and humidity levels at the interface between the patient and the surface to mitigate pressure ulcer risk factors. The objective of this study is to evaluate the effectiveness of ClimateCare® in improving wound outcomes and minimizing complications of pressure ulcers.

Methods: Patients with a stage III/IV pressure ulcer admitted for surgical closure were included in the randomized-controlled trial. All patients received the Fluid Immersion Simulation system (FIS), either with or without the ClimateCare® treatment based on a convenience sampling method. The subjects were monitored for 14 days post-closure (POD-14) for assessment of wound status and complications, including moisture, maceration, drainage, dehiscence, epidermolysis, necrosis, and demarcation.

Results: A total of 32 patients completed the study, where 18 patients received the ClimateCare® treatment and 14 patients did not. In the control group, 71% of patients had complications while 17% had complications in the ClimateCare® group (P = .001). In addition, 33% of patients without the ClimateCare® had open wounds, while no patients who received ClimateCare® treatment had open wounds (P = .011). Patient acceptability regarding treatment comfort, difficulty with mobilization, and pain at surgical site were not significantly different between ClimateCare® and control groups.

Conclusion: Our findings suggest that the ClimateCare® treatment in conjunction with the FIS may be effective in decreasing risk of post-operative complications and emphasize the importance of moisture control and pressure offloading in patients. Future studies should be conducted to characterize the effects of ClimateCare® in minimizing the risk of complications following wound closure.

P50  |  Reliability And Validity Of Skin Blot Examination For Adenosine Triphosphate To Detect Pressure-Induced Minor Tissue Damage

Ruka Inami1, Yoko Hasegawa1, Kazuhiro Ogai1, Katsunori Kato1, Nana Nuka2, Yoko Onishi2, Masaru Matsumoto2, Chizuko Konya2, Takeo Minematsu2
1Department of Bio-Engineering Nursing, Ishikawa Prefectural Nursing University, Kahoku, Ishikawa, Japan; 2Department of Adult Nursing, Ishikawa Prefectural Nursing University, Kahoku, Ishikawa, Japan

Background: Early detection of subtle tissue damage caused by external force is a promising approach for personalized pressure injury prediction. We previously reported that adenosine triphosphate (ATP) collected from compressed skin through skin blotting can identify tissue damage. The aim is to study the reliability and validity of ATP detection through skin blot examination for identifying minor tissue damage induced by pressure.

Methods: The intra-rater reliability was assessed by three examiners conducting an ATP test through skin blotting twice. This involved wiping the targeted skin area five times in one direction with a non-woven cloth moistened with distilled water and applying a saline-moistened nylon membrane for 10 minutes. Subsequently, the nylon membrane was placed into an inspection kit, and the amount of collected ATP was measured using a portable ATP analyzer. The intra-class correlation coefficient (ICC 1,1) was then calculated based on these two ATP measurements. For the validity study, 6-week-old male BALB/cCreSlc mice were acclimatized and housed for 1 week before being randomly assigned to the compression group (n = 5) and the control group (n = 5). The dorsal skin of the compression group underwent compression at 1000 mmHg for 4 hours. The ATP test using skin blotting was conducted on the skin surface of the compressed area and the surrounding skin. The ratio of ATP in the compressed area to the surrounding skin (compressed/surrounding skin) was compared using independent t-tests between the compression and control groups.

Results: The ICC1,1 was 0.615 (p = 0.004). Following compression, the compression group exhibited Grade I pressure ulcers. The ATP ratio (compressed/surrounding skin) was significantly higher after compression in the compression group (2.3 ± 0.4 vs 1.3 ± 0.6, p = 0.018).

Conclusion: The skin blot examination for ATP is a reliable and valid point-of-care method for detecting minor tissue damage in a mice model of pressure injuries.

P51  |  Skin Barrier Disruption Measured By Transepidermal Water Loss (Tewl) Correlates With Total Protein Recovered From Retained Medical Adhesive Test Articles

Olivia Reiff1, Madeline Hakala1, Walter R. Pedersen1, Tim Houser2, Tage Carlson1, Abram D. Janis3
1Hollister Incorporated, Libertyville, IL; 2Dermico LLC, Broomall, PA

BACKGROUND. A key metric of skin health and function is transepidermal water loss (TEWL), which increases when the integrity of the stratum corneum is compromised; TEWL is commonly used in clinical studies to measure the effects of repeated application and removal of medical adhesives. Disruption of the skin barrier can occur in people who have undergone ostomy surgery and must repeatedly apply & remove the adhesives used to affix ostomy pouching systems to the peristomal skin. Historically, skin barrier disruption demonstrated by TEWL has been observed to increase over sequential skin stripping events. However, our studies have been limited to observation of the
P52 | Pain Relief Efficacy Of Ibuprofen Releasing Foam Dressing On Outpatient Patient With Partial Thickness Burn Wound

Joon Hyuk Lee, Jun Ho Lee
Plastic surgery, Yeung Nam University, South Korea

Purpose: Pain management in burn treatment is important in improving wound healing and quality of life. Ibuprofen is a proven pain relieving agent in patients with partial thickness burn by intravenous injection. The purpose of this study is to evaluate the efficacy of Biatain Ibu® (polyurethane foam containing ibuprofen) in pain control for outpatients with partial thickness burns.

Methods: A prospective randomized clinical trial was performed in outpatients with partial thickness burn from August 1, 2017 to July 31, 2018. Acute pain, chronic pain, complications, days for re-epithelialization and patient’s satisfaction were compared between Biatain Ibu® and Biatain® groups.

Results: A total of 20 patients (Biatain Ibu®, n=10; Biatain®, n=10) were assessed in the trial. On Burn days 3, 5, 7, 11, 13, and 15, the acute pain levels were significantly lower in the Biatain Ibu® group than in the Biatain® group. Complications, chronic pain levels and days for re-epithelialization were not significantly different between the two groups. Patient’s satisfaction was not statistically significant but was higher in the Biatain Ibu® group.

Conclusion: Biatain Ibu® is effective in relieving pain in outpatients with partial thickness burn without decreasing patient satisfaction, wound healing ability or developing any complications.
A key cause of delayed healing in chronic wounds is the impaired cell migration due to systemic illnesses and advanced age. The current clinical care does not specifically address this impairment. Electrotaxis is the directional and accelerated cell migration guided by a direct current electric field (DC EF). Electrotaxis shows good efficacy in in vitro cell migration. But its in vivo efficacy is limited due to the difficulty in safely applying the EF strength typically used in in vitro studies (200 mV/mm) to in vivo tissues. Tissue damage can be caused by electrochemical reaction (ECR)-induced pH/temperature changes at high EF strengths. We developed a novel hydrogel ionic circuit (HIC) electrode to minimize pH/temperature changes when applying high-strength DC EFs. Our goal here was to determine the safety and in vitro electrotaxis efficacy of HIC electrodes when applying 200 mV/mm and higher DC EFs.

A HIC electrode consists of a carbon electrode inserted in a chamber filled with a saturated phosphate salt solution to absorb ECR-induced pH/temperature changes. The chamber is separated from the skin by a polyethylene glycol hydrogel, which prevents high-concentration phosphate salt ions from diffusing into the tissue. To evaluate the safety, 3 DC EF strengths (200, 400, and 800 mV/mm) were applied to pig skin for 5 hrs. Skin pH and temperature were measured right after EF application. An in vitro electrotaxis setup and HaCaT cells were used to test the electrotaxis efficacy. 200, 400, and 800 mV/mm were applied for 5 hrs. The directedness and projected migration speed along the EF direction of HaCaT were calculated. Directedness is a measure of cell migration direction, which is the cosine of the angle between the migration and the EF vectors. 3 repeats were used for safety experiments. 100 cells were analyzed in each migration experiment.

At all 3 DC EFs tested, HIC electrodes maintained a safe skin temperature below 42.8 °C and a safe skin pH between 5.9 and 6.9. But a conventional carbon electrode increased the skin temperature to 43.9 °C, 49.7 °C, and 53.0 °C at 200, 400, and 800 mV/mm, respectively. It changed the skin pH to 4.0 (anode)/10.7 (cathode) and 2.4 (anode)/13.1 (cathode) at 400 and 800 mV/mm, respectively. These pH/temperature changes can cause skin damage. At 200 mV/mm, HIC electrodes achieved similar directedness (0.67) and projected speed (11.1 μm/hr) as conventional carbon electrodes. At higher DC EFs, HIC electrodes significantly (P<0.05) increased the directedness to 0.90 and 0.95 and the projected speed to 33.2 and 61.8 μm/hr at 400 and 800 mV/mm, respectively.

In summary, we showed the ex vivo safety of HIC electrodes when applying 200 mV/mm and higher DC EF to skin tissues. We showed that high-strength DC EFs applied by HIC electrodes enhanced the directedness and projected migration speed of HaCaT cells compared to 200 mV/mm DC EF.
**P56** | **Evaluation Of An Ultrathin Synthetic Antibiofilm Matrix In The Healing Of Full Thickness Burn Wounds In A Porcine Model**

Eric Crawford, Naveen Nagiah, Gaurav Pranami, Ankit Agarwal  
Imbed Biosciences, Middleton, WI

**Introduction:** The healthcare costs associated with treatment of chronic and burn wounds exceeds $25 billion annually in the US. Here we report the evaluation of a synthetic matrix, made of polyvinyl alcohol with a polymeric multilayer coating impregnated with silver and gallium that together kill biofilm bacteria, in healing of full thickness porcine burn wounds. The matrix has been shown to kill $>4\,\text{Log}_{10}$ CFUs of clinically relevant planktonic and single-/multispecies biofilm bacteria (reported elsewhere).

**Method:** 20 full thickness burn wounds of 2 cm diameter were created on the back of 3 pigs using heated brass rod following a published method (Telgenhoff et al. 2007). Post burn, the wounds were excised and ten wounds on either side of the spine on each animal were treated with/without the matrix and wrapped with a protective Curad pad and ELASTIKON to secure the dressings. The dressings were reapplied on days 2, 4, 7, 10, 14 and 21. On days 0, 3, 7 and 28 blood samples were collected for complete blood count and quantification of silver and gallium in the blood plasma by inductively coupled plasma – mass spectrometry (ICP-MS). Samples for histology were collected on Days 7 and 28. After euthanasia, gross necropsy of all major organs were performed.

**Results:** Macroscopically and microscopically, wound healing followed the normal progression at all intervals and the wounds were healed completely by day 28. No major differences in the average scores for wound healing parameters -- including granulation tissue, erythema, edema and re-epithelialization -- at 7 and 28 days of treatment was observed. Histology analysis showed similar to near identical healing response scores at days 7 and 28. The matrix was classified as slight irritant when compared to Curad pads (control) at day 28, however this did not appear to affect wound healing, thus showing that the matrix is safe for use in full thickness burn wounds. Furthermore, the levels of silver and gallium in blood plasma assessed by ICP-MS were found to be below the limit of detection (0.5 ppb), thus confirming that there was no systemic absorption of silver or gallium during the 28-day healing period from the repeated topical applications of the matrix.

**Conclusion:** The antibiofilm silver/gallium matrix did not impair the healing of full thickness thermal burn wounds in a preclinical porcine model, exhibiting a high potential for clinical use.

**P57** | **Estetrol Dampens Inflammation And Accelerates Wound Repair In Vitro And In Vivo**

Leah Cooksey$^1$, Alexandria Kidd$^1$, Alexander Johns$^1$, Celine Gerard$^2$, Matthew Hardman$^4$, Holly N. Wilkinson$^1$

$^1$Biomedical Institute of Multimorbidity, Centre for Biomedicine, Hull York Medical School, Hull, United Kingdom; $^2$Mithra Pharmaceuticals, Liège, Belgium

Chronic non-healing wounds, which primarily affect the elderly and diabetic, remain a significant area of clinical unmet need. In ageing women, the rapid loss of circulating 17β-estradiol (E2) post-menopause contributes to skin structural decline and delayed wound repair. Studies over 20 years ago first demonstrated the importance of E2 in reversing age-related delayed wound healing. Since then, E2 has been linked to a diverse range of wound cell processes, yet the risk of off-target effects has hampered development of E2-mediated therapies for clinical use. More recently, estetrol (E4), a natural estrogenic steroid produced exclusively by the human fetal liver during pregnancy, has been suggested as a promising alternative to E2 due to its more favorable safety profile. E4 is the estrogenic component of a recently approved combined oral contraceptive and is currently in late-stage clinical development as a hormone replacement therapy. However, no studies to date have investigated the effects of E4 on wound repair.

The primary aim of this study was to determine the effect of E4 on multiple aspects of wound healing. Here, E4 significantly increased scratch wound closure in fibroblasts and keratinocytes at comparable levels to E2, while the pro-migratory effects of E4 were also conserved in senescent fibroblasts. Similar to E2, E4 demonstrated substantial anti-inflammatory properties, significantly reducing MMP2 activity in fibroblasts and dampening the expression of pro-inflammatory markers in M1-polarized macrophages. Moreover, E4 effects appeared to be regulated by both ERα and ERβ in a cell type specific manner. Finally, topical administration of E4 in an LPS model of delayed wound healing reduced inflammation and significantly accelerated wound closure. Collectively, these pre-clinical data show that E4 mediates multiple stages of healing, supporting future clinical investigation of the impact of E4 on delayed wound healing in the elderly.

**P58** | **Selective Depletion Of S. Aureus Restores The Skin Microbiome And Accelerates Tissue Repair Following Injury**

Holly N. Wilkinson$^2$, Amber Stafford$^1$, Michelle Rudden$^1$, Nina Rocha$^1$, Alexandria Kidd$^1$, Andrea Bell$^2$, Jeffrey Hart$^2$, Christian Röglin$^2$, Bob De Rooij$^2$, Matthew Hardman$^1$

$^1$Centre for Biomedicine, University of Hull, Hull, East Riding of Yorkshire, United Kingdom; $^2$Cica Biomedical Ltd, Knaresborough, United Kingdom; $^3$Micreos Pharma, Bilthoven, Netherlands

Our skin is home to a diverse community of commensal microorganisms that are integral to cutaneous function. Microbial dysbiosis and barrier perturbation increase the risk of local and systemic infection. *Staphylococcus aureus* is particularly problematic with high levels of antimicrobial resistance and direct association with poor healing outcome. Thus, innovative approaches are needed to selectively kill skin pathogens, such as *S. aureus*, without harming the resident microbiota. Bacteriophage-derived cell wall-lytic enzymes, known as endolysins, are emerging as promising alternatives to traditional antibiotics. However, their efficacy is seldom assessed in models harbouring a complex multifactorial wound microenvironment.
microbiome due to the historic challenges associated with bacterial sampling, characterisation and profiling. We thus developed a novel pipeline, combining long-read metagenomic sequencing and RNA-sequencing, to provide the first demonstration that endolysin selectively inhibits endogenous *S. aureus* in vivo, leading to higher microbial diversity and promoting multiple aspects of wound repair. Further mechanistic evaluation confirmed the importance of microbiome modulation for effective healing in human skin. Together, these findings provide new insight into the role of *Staphylococcus* in healing pathology, and support further therapeutic development of *S. aureus*-targeted endolysins for clinical management of skin and wound infections.

**P59 | Antimicrobial and Antibiofilm Performance Of An Ultrathin Synthetic Matrix Containing Silver And Gallium**

Naveen Nagiah, Eric Crawford, Gaurav Pranami, Ankit Agarwal
*Imbed Biosciences, Middleton, WI*

**Introduction:** Biofilms are implicated in delayed healing in chronic wounds, however there is no commercially available topical formulation effective in dispersal of biofilms in wounds. Here we report the evaluation of antimicrobial, antimicrobial barrier and antibiofilm performance of a synthetic matrix, made of polyvinyl alcohol with a polymeric multilayer coating impregnated with silver and gallium, both in vitro and in vivo.

**Methods:** Antimicrobial performance of the matrix over 24 and 72 h was tested against 8 clinically relevant microbes (*K. pneumoniae, E. coli, C. albicans, C. tropicalis, MRSA, S. aureus, P. aeruginosa, and E. faecalis*) per ISO 22196. Its antimicrobial barrier performance was evaluated by pipetting 10 μL of bacterial inoculum of *P. aeruginosa, A. baumannii* or *K. pneumoniae* on the matrix placed on an agar plate and incubating at 37 ± 2 °C for 3 days and quantifying the CFU subsequently.

To evaluate the antibiofilm performance in vitro, robust biofilms containing 10^8 CFU of *A. baumannii* or *K. pneumoniae* were established on gauze specimens over 48 h and rinsed with saline to remove planktonic bacteria. Moist biofilm specimens were then treated with a single application the matrix for 24 h, and the CFUs were determined relative to no-treatment controls. In vivo assessment was performed by transplanting preestablished biofilm in 1 cm dia. full thickness porcine wounds by placing the gauze specimens supporting 10^8 CFU *P. aeruginosa* biofilms for 24 h. Afterwards, the gauze specimens were removed and wounds were treated with the matrix once daily for two days. On day 3, biopsies of all wounds were minced and CFUs were determined relative to no-treatment control.

All assays were carried out with at least three replicates for each sample. Groups were compared using a Student’s t test at P < 0.05.

**Results:** The matrix killed > 4 Log_{10} CFUs of all microbes tested per ISO 22196, thus confirming its antimicrobial activity against planktonic bacteria. It also killed bacteria on its surface and prevented the breakthrough of microbes over 72 h thereby demonstrating its ability to serve as an antimicrobial barrier. > 4 and 1.5 Log_{10} CFU reduction of *A. baumannii* and *K. pneumoniae* biofilms bacteria was achieved in vitro over 24 h with a single application of the matrix, and the matrix was killed 1.5 Log_{10} CFU of *P. aeruginosa* biofilm bacteria in vivo, thus establishing its antibiofilm performance.

**Conclusion:** The silver/gallium matrix is effective in killing both planktonic and biofilm bacteria and is thus suitable for assessment in the treatment of biofilms in chronic wounds.

**P60 | Exploring The Potential Of Collagen Scaffolds In Calvarial Bone Regeneration**

Leya Groysman, Jenn Park, Fernando Arias, Adrienne Nemchik, Alexandra Verzella, Roberto Flores, Piul S. Rabbani
*Plastic Surgery, NYU Grossman School of Medicine, Brooklyn, NY*

Autologous bone grafts are widely used to repair bone defects, such as craniofacial abnormalities. However, procedures involving alveolar bone grafts are invasive and there is risk for donor site infection and morbidity. Bone tissue engineering, which incorporates stem cells, scaffolds, and biological factors, is a promising alternative to current repair methods for cleft palate and other bone defects. In our study, we assess the impact of several scaffolds, including collage sponge implant, collagen gel with superficial silicone sheet, and collagen gel only on bone regeneration in a calvarial defect. Natural polymers, such as collagen, can be a useful material in bone tissue engineering. At 5 weeks of age, we created a 3 mm diameter calvarial defect in the parietal bone of mice. We implanted a collagen sponge pre-loaded with 20 microliters of PBS, a collagen gel with a silicone sheet, collagen gel only, or no intervention at all (control). At 4 or 12 weeks, we harvested the mice calvaria for analysis. We performed 3D-reconstruction volumetric analysis using the Amira software. For immunostaining, we cryosectioned the bone samples at a thickness of 30 μm. We used immunostaining to characterize the components of the osteoconductive environment. At 4 and 12 weeks, collagen gel implant with overlying silicone sheet had the highest percent of bone growth in the defect, 24.7% and 30.9%, respectively. Collagen gel and collagen sponge implants had similar percentages of bone growth at 12 weeks, 25%, and 25.6%, respectively. All scaffold implants had a higher percentage of bone growth in the defect compared to the control condition (2% at 4 weeks and 7.8% at 12 weeks). Immunostaining analysis for the collagen gel and silicone sheet implants showed endomucin and CD31 staining for blood vessels within the fibrous bridge that connects the newly generated bone in the defect area to the surrounding intact bone. We also observed endomucin and CD31 signal in the periosteum at the edge of the defect and surrounding the new bone marrow. The superior bone growth at 4 and 12 weeks in the collagen gel and silicone sheet condition suggests that these materials foster an enhanced osteoconductive environment. The prominent presence of endomucin and CD31 staining marks neovascularization, a vital component for sustainable bone regeneration, and continuity between new and intact bone, across the fibrous bridge and into the bone marrow.
intact bone. In the future, understanding the mechanisms involved in bone regeneration, and identifying key cells, can help optimize scaffold treatment options for bone defects for applications in bone tissue engineering procedures in pediatric cases, such as cleft lip and palate disorders.

P61  |  Development of a Benchtop Pelvic Model for Iterative Design of a Self-Fitting Vaginal Stent

Ashley Hicks¹, Tochukwu Ozulumba², Julie C. Hakim², Elizabeth Cosgriff-Hernandez¹
¹Biomedical Engineering, University of Texas at Austin, Austin, TX;
²Obstetrics and Gynecology, Baylor College of Medicine, Houston, TX

Purpose: The goal of this study was to develop a benchtop vaginal model to test self-fitting polymer stents for mitigating vaginal fibrosis.

Methods: We used an emulsion templating approach to fabricate a thermoresponsive shape memory polymer (SMP) foam from poly(e-caprolactone). To expedite the design of the stent geometry and provide better predictions of performance in vivo, a custom benchtop pelvic model was developed to simulate vaginal anatomy, temperatures, and pressures. Clinical MRI images were used to construct a silicone vaginal canal from EcoFlex. The canal was encased in an acrylic pressure chamber to simulate pelvic floor contractions. Physiological temperatures were replicated by wrapping heating tape (BriskHeat, 144W) around the pressure chamber. Model parameters were validated against clinically reported values. We also tested the usefulness of our testing apparatus in iterative device design by modeling stent deployment and retention. Stent expansion and deformation was visualized using a hysteroscope (Endosee) placed near the introitus of the model.

Results: Temperature readings were taken at three locations within the model (cervical, central, and introitus regions) in triplicate and confirmed to be within physiological ranges (36°C ± 1°C). A perineometer (MizCure, OWOMED) was used to assess the radial forces within the model according to a previously established protocol. The model pressures were measured to be within physiological parameters (19 mmHg ± 1 mmHg). A crimped SMP vaginal stent expanded to walls of the canal (~70% increase in cross-sectional area) in <5 minutes after irrigation with warm water (~45°C). The stent’s cross-sectional area decreased by <1% in response to physiological pressure. Stent diameter exhibited ~8% decrease along the anterior-posterior dimension, with a corresponding 8% increase distally.

Conclusion: Overall, these results demonstrate the potential of this newly designed SMP foam for use as a self-fitting vaginal stent and provide a benchtop pelvic model for guiding the design of gynecological devices.

References:

P62  |  The Effects of a Copper-Iodine Complex Solution on the Reduction of Biofilms Grown on Implant Materials and In Vivo Porcine Wounds

Julian Bejarano², Jeffrey M. Lawrence², Steven J. Kavros¹
¹Vascular Surgery Associates, Minneapolis, MN; ²Gunderson Lutheran, Viroqua Center for Orthopedic Surgery, Viroqua, WI; ³Clyra Medical Technologies, Westminster, CA

Introduction: Copper-Iodine Complex Solution (CICS) is an FDA 510 (k) cleared medical device as a wound irrigation system. This unique complex has the capacity to neutralize a broad number of pathogens such as bacteria, viruses, yeast, and fungi without evoking bacterial resistance. Free Iodine (I2) is a recognized powerful and broad-spectrum antimicrobial with no known resistance by exhibiting multi-mechanisms of action, highlighting: (i) penetration into the cell wall of the microorganism, causing blocking of the hydrogen bonds which results in damage to the phospholipid cell membrane, (ii) and damage and denaturing of the essential proteins, nucleotides and fatty acids by binding to thiol and amine groups, leading to rapid cell death. Free iodine acts as a preservative agent that helps to remove contamination within the CICS for effective wound cleaning. CICS has been proven to be non-cytotoxic, non-pyrogenic, non-irritating, and non-sensitizing to dermal tissue. The purpose of this study is to quantitatively evaluate the effect of CICS on biofilm in a porcine model and commonly used implant material substrates (silicone and titanium alloy).

Materials and Methods: Two implant materials were used in this study to grow biofilms, silicone, and titanium alloy substrates. Three independent time trials were conducted, 5h, 24h, and 72h. Data was analyzed with one-way Anova and Tukey post-hoc tests. p-value: <0.05. This in vitro biofilm test was conducted by the Center for Biofilm Engineering at Montana State University.

Results: 1. Efficacy of CICS against S. epidermidis mature biofilms on silicone substrate

Results: 1.7 log reduction at 30 min, 4.7 log reduction at 2 hours, 6.6 log reduction at 5 hours and 7.0 log reduction at 24h and 72h. No colonies observed at 24h and 72h.

No statistical difference was observed between 5h, 24h and 72h kill rates.

2. Efficacy of Bioclyne against S. aureus mature biofilms on titanium alloy substrate

Results: 0.6 log reduction at 5 min, 1.8 log reduction at 0.5 hours, 4.7 log reduction at 2 hours and 7.5 log reduction at 24h. No colonies were observed at 24h.

GLP in vivo study (porcine model) to assess the anti-biofilm and antimicrobial activity.

Results: CICS reduced total bacteria in the biofilm by 2.0 – 2.5 log CFUs compared to initial inoculation level.

Conclusion: Copper-Iodine Complex Solution has been shown to generate a significant log reduction in the growth of both Staph aureus and staph epidermidis biofilms grown on silicon and titanium implant materials. Biofilms were also reduced in a in vivo wound porcine model. Further studies are needed to show that this can help to prevent and to treat infected implants in humans.
Introduction: Copper-Iodine Complex Solution (CICS) is an FDA 510 (k) cleared medical device as a wound irrigation system. CICS is indicated in wound management, cleansing, irrigating, moisturizing, and debriding of acute and chronic dermal lesions that are partial or full thickness wounds. This unique complex has the capacity to neutralize a broad number of pathogens such as bacteria, viruses, yeast, and fungi without evoking bacterial resistance1-4.

CICS has been proven to be non-cytotoxic, non-pyrogenic, non-irritating, and non-sensitizing to dermal tissue12-16.

The purpose of this study is to quantitatively evaluate the effect of Copper-Iodine Complex Solution on bacteria, yeast, fungi, and SARS-CoV-2 virus in an in vitro model.

Materials and Methods: Trial #1 - demonstrates antimicrobial efficacy testing as a preservative in solution using five common organisms at 14 and 28 days.

Trial #2 - addresses time - kill data against 15 clinically relevant pathogens.

Trial #3 - addresses persistent antimicrobial efficacy after re-inoculation using 3 different time points.

Trial #4 - addresses and validates the efficacy of CICS against SARS-CoV-2.

Results: The results of all 4 independent in vitro studies will be reviewed in detail. There is significant log reduction of bacteria, yeast, and fungi in all in vitro evaluation. This is also evident in a variety of time periods that the organisms are exposed to CICS with the associated log reductions of clinical significance. Long-lasting CICS efficacy against ESKAPE pathogens (Enterococcus faecium, Staphylococcus aureus, Klebsiella pneumoniae, Acinetobacter baumannii, Pseudomonas aeruginosa, and Enterobacter sp) and Candida albicans and Candida tropicalis has been demonstrated up to 3 days. The final study shows the results of CICS against SARS-CoV-2 virus. After incubation with undiluted CICS for 10 minutes, viral titers dropped by 2 logs (one tailed t-test p-value = 0.0140). After incubation with undiluted CICS for either 30 minutes or 60 minutes, viral titers dropped below the limit of detection (< 75 TCID50 per ml).

Conclusion: Copper-Iodine Complex Solution has been shown to create a significant log reduction with kill rate in multiple gram positive and gram-negative bacteria, yeast, and fungi. Additionally, CICS has been shown to be effective against SARS-CoV-2 virus. Further studies are needed to support these findings.

Materials and Methods: The NP-PWD is a transparent, single component NPWT dressing to deliver NPWT that does not need a foam or gauze to function. The purpose of this study is to introduce Negative Pressure Platform Wound Device (NP-PWD).

Materials and Methods: The NP-PWD is a transparent, single component NPWT dressing that consists of an impermeable polyurethane membrane. It has a permeable adhesive base which is attached to the perimeter of the wound, enabling fast Band-Aid-like application. The suction pump is connected to the underside of the membrane with tubing. The inner surface of the PWD contains pyramid-like structures protruding toward the wound. Once the suction pump is turned on and the desired negative pressure is achieved, the embossed membrane is pulled into contact with the entire surface area of the wound and the space between the pyramids is providing channels for even distribution of negative pressure as well as for exudate removal. Folds in the membrane provide secondary channels for negative pressure and fluid removal. The NP-PWD has been extensively validated in preclinical large animal models as well as in clinical case series.

Results: The results have demonstrated that the NP-PWD can function effectively at lower negative pressures (~80 mmHg and ~50 mmHg) promoting healing, reducing tissue necrosis, inflammation and bacterial burden in the wounds. Importantly, when compared to the conventional devices, with foam or gauze, no differences were observed. Clinical studies have reported that patients tolerate the NP-PWD well. In addition, the possibility to monitor the wound without dressing removal has proven to be beneficial in a clinical setting.

Conclusions: The NP-PWD is a simplified, single component NPWT system eliminating the use of the filler material that commonly causes challenges during treatment.
Pathological biofilm formation is a major issue that restricts the functional use of implants and scaffolds in wound healing, resulting in delayed healing, chronic infections and impaired tissue regeneration. Embedding nanoparticles directly into the wound-bed could be a viable strategy, but present the drawback of quick diffusion. It also doesn’t provide enough mechanical support for the healing tissue. A solution to this problem would involve embedding the particles inside a wound dressing biomaterial. Introducing antibacterial nanoparticles into woven natural biomaterials as biofabrics would help retain antibacterial activity while providing mechanical support. Here we propose to develop a controlled release antibacterial biofabric produced by electrospinning polymethyl methacrylate (PMMA) as the base material, silk fibroin (SF) as a component to provide mechanical strength, and cationic hyperbranched polyethyleneimine (PEI) to provide antibacterial properties. We propose the antibacterial biofabrics could be a valuable tool to treat chronic wounds as well as coatings for implants (i.e. cardiac pacemakers) that potentially cause infections.

**Methods:** SF Regeneration: Silkworm cocoons are boiled to separate fibroin yarns. Silk fibroin is solubilized in LiBr solution followed by dialysis to achieve SF solubility in water. Electrospinning: In order to make biofabrics, PMMA, SF, and/or PEI were prepared and placed into a 5 ml solution of nutrient broth for an antibacterial assay, alongside samples of 5 mg SF particles in nutrient broth and nutrient broth alone. *P. aeruginosa* infections but also will provide mechanical support via antibacterial properties. We propose the antibacterial biofabrics could be a valuable tool to treat chronic wounds as well as coatings for implants (i.e. cardiac pacemakers) that potentially cause infections. **Purpose:** To determine if there are differences in strain relief in sacral dressings under physiological loads. **Background:** Patients who must remain prone for long periods end up placing a high burden on the skin of the sacrum. A class of border dressings has been in use to mitigate the lateral strains on the sacrum while the patient shifts while the sacrum is under load. There a several “substantially equivalent” devices on the market, but their relative performance in strain mitigation is unknown. **Methods:** A custom digital image correlation system with a bead-loaded silicone sheet was used to monitor the strains in the sheet under physiological loads (Mimura et al. 2009 WRR., 155 mmHg). A stepper motor was used to apply 216 N of external shear force in 0.625-mm steps for 40 steps. The 4 dressings were compared to no treatment in triplicate. An initial measurement of the maximum gross lateral strain was quantified in Fiji and compared by one-way ANOVA (a = 0.05) followed by a pairwise-Tukey HSD post-hoc test. **Results:** The mean of the dressings maximum strains were 0.0162, 0.0231, 0.0285, & 0.0267 (nil = 0.1206). The ANOVA revealed very significant differences (p = 1.09 x 10^-13). All dressings were very substantially better than nil (p < 0.00009). The best performing dressing was better that all the rest (p < 0.0131). The second best was better than the remaining 2 (p < 0.049).

**Conclusions:** We have found a means to compare regulatory similar sacral dressings under physiological loads and find statistically significant differences in strain relief. Additional work continues to identify the pressure load at which performance among the dressings begins to differ.

**P67 | Effectiveness of Using Proheal-Wrapped Nasopore for Nasal Bone Fracture Surgery**

Young-Joon Jun  
plastic surgery, The Catholic university of Korea, Seoul, Other, Korea (the Republic of)

**Aim:** In nasal bone fracture surgery, the post-operative packing material can be divided into conventional materials, such as Vaseline gauze that requires removal, and absorbable materials that is totally degraded and does not require removal. Nasopore, a biodegradable synthetic polyurethane foam, is the material mainly used as nasal dressing. Although it has no need for post-operative removal, it is soft and hydrates quickly, making it difficult to provide sufficient support to maintain the post-reduction status. The aim of this study is to introduce a novel method to improve durability of Nasopore with Proheal.

**Method:** Instead of packing Nasopore directly into the nasal cavity, we wrapped Nasopore with Proheal, which is a collagen wound dressing material. After reduction of the nasal bone, nasal cavity was packed with nasopore wrapped with proheal (Fig.2), while the non-fractured nasal cavity was packed with proheal rolled up only.

**Results/Discussion:** As Proheal help delay the hydration of Nasopore, it improves supportability and maintenance of Nasopore (Fig.4). Additionally, nasal mucosal healing was observed to be faster, indicating a potential positive impact of proheal on the healing process.
**Conclusion**: The proheal-wrapped nasopore provided sustained support and exhibited a longer-lasting property compared to nasopore alone. Moreover, it contributed to faster nasal mucosal healing. These findings suggest that the application of proheal-wrapped nasopore could be a superior choice in nasal bone fracture surgery, as it offers improved stability and facilitates a quicker recovery. Considering proheal-wrapped nasopore may lead to enhanced surgical outcomes and improved patient recovery.

**P68 | Representation Of Patients With Renal Disease In Wound Healing Intervention Studies**

Kirtana Sandepudi, Krish Shah, Kristin Huffman, Sobhi Kazmouz, Nishanth Sadagopan, Rachel Donaldson, Robert Galiano
Department of Plastic and Reconstructive Surgery, Northwestern University Feinberg School of Medicine, Chicago, IL

**Background**: Patients with renal failure and chronic kidney disease (PWRD) have profoundly impaired wound healing due to various factors including comorbid arterial disease, poor nutrition, uremia, anemia, and neuropathy. Diabetic foot ulcers (DFUs) are a common chronic wound seen in this population. PWRD also have higher risk of calciphylaxis, a rare but highly morbid wound caused by uremic arterial calcification. Despite these factors, PWRD are commonly excluded from wound healing studies. The purpose of this review is to quantify the representation of PWRD in studies on DFU and lower extremity wound healing interventions, with little improvement over the last 10 years. The few papers that do compare outcomes between patients with and without renal disease continue to suggest existing interventions are not effective in PWRD. There remains a dire need for investigation into wound healing treatments for these patients.

**Methods**: A systematic review was conducted using PRISMA guidelines to investigate wound healing interventions for treatment of DFUs or calciphylaxis. PubMed, Cochrane, Embase, MedLine, and WebofScience databases were queried for relevant articles published from February 2013-2023. Duplicates were eliminated, and abstract and full text review were conducted. The primary outcome was inclusion of PWRD. Secondary outcomes included number of PWRD and specific wound healing parameters.

**Results**: The initial database search yielded 688 articles. After abstract and full text review, 82 papers were analyzed. Of these papers, only 30 (37%) included PWRD and reported the number of patients with this comorbidity. 34 papers (40%) explicitly excluded PWRD. While the remaining 18 papers did not exclude PWRD, they did not explicitly report patient comorbidities. Thus, inclusion of PWRD could not be determined. Among DFU papers, 2.8% of patients represented were PWRD. However, previously reported prevalence of renal disease among DFU patients ranges from 15-50%. For calciphylaxis papers, 55% of included patients were PWRD. There was no significant difference in representation of PWRD in 2013-2017 versus 2018-2023 (p=0.75).

Only 4 papers, including one case report, compared outcomes between PWRD and patients without. Two papers on surgical interventions showed poorer results in PWRD. Chou et al. studied free flap transfers for DFU treatment and noted that 90% of failed flaps belonged to PWRD. Walters et al. investigated split-thickness skin grafts for DFUs and found that patients with slow-healing plantar ulcers were more likely to have renal disease (p=0.002).

**Conclusion**: PWRD experience delayed wound healing due to several factors. However, they are not well-represented in literature on wound healing interventions, with little improvement over the last 10 years. The few papers that do compare outcomes between patients with and without renal disease continue to suggest existing interventions are not effective in PWRD. There remains a dire need for investigation into wound healing treatments for these patients.

**P69 | Macrophage-Derived Extracellular Vesicles Promote Wound Closure By Regulating Keratinocyte Proliferation Via MIR-425**

Dong Jun Park1, Wooil Choi1, Sakeef Sayeed1, Kayla Ho1, Jenny Kezios1, Robert Dorschner2, John Nolan3, Brian P. Eliceiri1
1Surgery, University of California in San Diego, San Diego, CA; 2Dermatology, University of California San Diego, San Diego, CA; 3Scintillon Institute, San Diego, CA

Small extracellular vesicles (EVs) are released by cells and deliver biologically active payloads important in the intercellular signaling that coordinates the response of multiple cell types in cutaneous wound healing. Here we used a cutaneous injury in mice as a physiologically relevant donor of pro-reparative EVs to treat impaired wound healing. We established a functional screen for miRNAs that increased the pro-reparative activity of EVs and identified miR-425-5p as a mediator of keratinocyte proliferation. Based on the abundance of macrophages identified by transcriptomic profiling of the EV donor site, we tested transgenic mice expressing a tetraspanin CD9-GFP fusion protein under the control of a macrophage-specific promoter. These studies showed that a population of macrophage-derived EVs was internalized by dermal fibroblasts to regulate the proliferation of underlying keratinocytes that is mediated in part by miR-425-5p and supports a key role for EVs in mediating pro-reparative intercellular signaling.

**P70 | Biomaterials For Controlled-Release Of Agents With Antimicrobial Properties**

Charles Rice
Chemistry and Biochemistry, University of Oklahoma, Norman, OK

**Introduction**: Our compound, PEG-BPEI, counteracts (i) pathogens, (ii) biofilms, and (iii) toxins using electrostatic binding with the anionic components of each target. This occurs simultaneously and independently; and these multi-factor benefits are unlikely to be found with other compounds. Our research team, funded by the NIH, Department of Defense, and the University of Oklahoma, has carried out a
Product Development: PEG-BPEI also differs from existing technology because it is not a peptide and thus resists proteolysis, unlike cationic peptides and peptide mimetics susceptible to rapid proteolytic degradation and/or protein binding. PEG-BPEI is a hydrophilic molecule that is completely miscible with water. We have demonstrated the ability to formulate PEG-BPEI with gels, creams, and polymers. We have used PEGylation to reduce the in vivo toxicity while retaining activity. Importantly, we also reduce toxicity issues by using very-low molecular-weight BPEI (600 Da) rather than higher molecular-weight BPEI (over 25,000 Da).

Methods: PEG-BPEI is cationic and uses electrostatics for binding with anionic sites on Gram-positive and Gram-negative bacteria. PEG-BPEI and the bioactive moiety 600 Da BPEI, have broad-spectrum activity to counteract (1) antimicrobial resistance (AMR) caused by the Gram-negative LPS layer; (2) AMR caused by Gram-positive cell wall and teichoic acids (3) AMR caused by metallo-β-lactamases; (4) Release of pro-inflammatory cytokines in response to the Gram-negative pathogen associated molecular pattern molecules (PAMPs) LPS and peptidoglycan; (5) Release of pro-inflammatory cytokines in response to the Gram-positive PAMPs teichoic acids and peptidoglycan; and (6) Biofilms formed by Gram-negative pathogens and; and (7) Biofilms formed by Gram-positive pathogens.

Results: Checkerboard assays using microtiter plates demonstrate the antibiotic properties. Growth curves demonstrate bacteriostatic effects. Scanning electron microscopy (SEM) was used to confirm that the combination treatment leads to abnormal morphology. Data collected with isothermal titration calorimetry and fluorescence spectroscopy demonstrate a mechanism of action. ELISA was used to neutralize endotoxins (LPS, LTA, peptidoglycan) that otherwise stimulate pattern recognition receptors, leading the release of pro-inflammatory cytokines. Additional data show that PEG-BPEI can be absorbed onto, and released from, drug-delivery systems

P71 | Review and Evaluation of AI-Based Algorithms for Wound Assessment and Decision Support

Fateme Fayyazbakhsh1, Niloofar Zendehdel1, Haodong Chen1, Lisa Gould2, Ming Leu1
1Mechanical and Aerospace Engineering, Missouri University of Science and Technology, Rolla, MO; 2Department of Surgery, South Shore Hospital, South Weymouth, MA

Chronic wounds are a prevalent global health concern, annually affecting 6.5 million Americans with $25 billion burden on healthcare systems. Accurate and timely wound assessment is crucial for effective wound management, enabling clinicians to monitor healing progress, identify complications, and optimize treatment strategies. However, traditional wound assessment methods and manual measurements are often subjective, qualitative, time-consuming, and prone to errors, leading to suboptimal wound care outcomes and prolonged healing times. Artificial intelligence (AI) based wound assessment tools have emerged as promising solutions to address these challenges. These tools utilize machine learning algorithms like deep learning and convolutional neural networks to analyze wound images and provide objective, quantitative assessments of wound characteristics, such as wound area, tissue type, and healing status. A growing body of research has explored the development and application of AI-based wound assessment tools. The objective of this work is to review the current landscape of AI-based wound assessment tools and decision support systems, encompassing both smartphone apps and research papers, aiming at assessing their accuracy, identifying knowledge gaps, and informing future research directions in this field. We assessed performance metrics, input information, AI methods employed, accuracy & speed of assessment, level of user intervention, dataset size and quality, and clinical evaluation. We found that most of these studies focused on wound documentation and development of image-based wound area measurement algorithms like thresholding, while more recent studies focused on developing algorithms using convolutional neural network and region-based segmentation for tissue classification, with relatively small datasets ranging from 80 to 600 wound images. While most of the studies utilized wound pictures taken by smartphones, more recent publications focus on measuring the wound depths using RGB-D cameras and other medical imaging devices. A few works delved into developing decision support systems, aiming at providing clinicians with enhanced decision-making capabilities by leveraging AI algorithms to analyze complex wound data and offer personalized recommendations. Our findings also revealed that the current body of literature and existing applications suffer from the absence of reliable datasets, inaccurate measurements, and need for operator intervention. Additionally, there exists a knowledge gap in volumetric wound assessment and decision support systems. As the body of literature expands and technology progresses, there is a growing need for AI-based wound assessment tools to transition from research labs to clinical applications.

P72 | Successful Management Of Diabetic Venous Ulcer With Novel Anti-Microbial Hydrogel

Margaret Ganey2, Timothy Ganey1
1BonePharm, LLC, Tampa, FL; 2Floraseptic, TAMPA, FL

Methods: Patient T is a 69-year-old male patient living at high elevation in Arizona who had developed a chronic diabetic venous wound on the lower leg. Despite being a professional trauma nurse in Emergency Room Medicine for over 45 years, persistent wound status for 12 months resulted in self-referred consideration of an antimicrobial hydrogel that has been shown to effect healing in diabetics (Figure 1).
The nurse-patient self-administered hydrogel once weekly until healed. Despite achieving complete and cosmetic closure after 9 weeks, Patient T accidentally reopened the wound tripping which resulted in a new deep ulcer abrasion (Figure 2). The patient followed a similar mode of treatment with self-administered hydrogel once weekly for six weeks at which time the wound was completely healed. Both wounds were treated with use of novel anti-microbial hydrogel and healed completely with full closure and acceptable cosmesis despite diabetic status and 12 months of open wound.

**Results:** Patient T followed protocol for weekly administration of **FloraSeptic** and documented the healing progress with weekly images. **FloraSeptic** contributed to the accelerated healing process in Patient T. All evidence of the diabetic venous ulcer wound showed closure by the end of the 30-day treatment period full closure of the wound signifying successful wound healing. (Figure 1) The second wound, or the reopened wound abrasion, showed similar closure in less than 2 months. Granulation tissue formation was observed within the first week of treatment, followed by a progressive reduction in wound size, improved tissue quality, and full closure, signifying successful wound healing. (Figure 2)

**Conclusion:** The application of FloraSeptic led to expedited wound healing, with complete closure. One important differentiating facet of the application was treatment for a persistent, non-healing wound in a patient with diabetes over a relatively short course with no issues of delay, seeping, or need for antibiotics. **FloraSeptic**, a novel and innovative wound care product, appears to offer a promising solution for healthcare providers in the management of challenging venous ulcers even amidst the additional complication of diabetes. Developed with a botanical formulation to promote healing, **Floraspetic** provides antimicrobial protection, and sustains a balanced and optimized pH that supports epithelialization and wound closure during the healing process. Previous evaluations in spine surgery have demonstrated reduced surgical site infections and accelerated healing, while at the same time retaining normal skin pigmentation.

**References:**

FloraSeptic, BonePharm, LLC, Tampa, FL


---

**P73 | Wound Management Of Unresectable Basal Cell Carcinoma Of The Scalp**

**Danial Qadir, James Basset, Amulya marellapudi, Jerrin George, Richard Simman**

*Plastic Surgery, University of Toledo College of Medicine, Toledo, OH*

Basal Cell Carcinoma (BCC) is the most common neoplasm in the United States, with sunlight exposure being the most common risk factor [1]. We present the unique case of a 52-year-old male presenting with advanced and locally invasive BCC affecting the scalp and nose. The carcinoma measured 18cm² on the nose and 390cm² around the scalp, with varied discoloration and draining. The patient suffered from severe pain, necrotic tissue, and an unexpected complication of maggot infestation within the wound and skull. Given the disease's advanced stage and extensive involvement, Moh’s micrographic surgery and other traditional surgical methods of resection for definitive treatment were deemed impractical. Instead, the patient was initiated on vismodegib, an oral chemotheraphy agent targeting the Hedgehog pathway, along with wound management strategies. Despite these efforts, only modest improvement in the affected areas was observed, delaying further discussions regarding surgical intervention. While surgical excision remains the gold standard for localized BCC, targeting signaling pathways like the Hedgehog pathway has emerged as a promising approach for challenging cases. The limitations in conventional surgical options due to the extensive involvement prompt consideration of advanced reconstructive techniques such as free muscle flaps, contingent upon the disease’s manageability and the patient’s candidacy. This case highlights the complexity in managing advanced BCC and the importance of palliative wound management for cases where surgical management is delayed or cannot be performed. Palliative wound care focuses on comfort rather than optimal healing strategies and includes management of pain, bleeding, odor, and exudate [2]. While inhibition of gene signaling pathways showed some benefit, further investigation and long-term follow-up are essential to assess the efficacy of such treatments in complicated BCC cases. Additionally, addressing unexpected complications like maggot infestation necessitates specialized interventions for control and prevention. In conclusion, this case emphasizes the need for a comprehensive understanding of various treatment modalities and warrants continued research to determine optimal wound management strategies for complex BCC presentations.

**References:**


---

**P74 | Clinical Applications of Wearable Electrocutical Devices for Chronic Wound Healing: A Systematic Review**

**Dhruv Seshadri, Ali Mutah, Joseph Amitrano**

*Lehigh University, Easton, PA*

**Background:** The need to optimize the use of electrocutical therapy for the site specific repair of chronic wounds has received tremendous attention in the last 20 years. Devices for chronic wound healing utilizing direct current, pulsed, or alternating electric current have been explored to stimulate the body’s cellular and molecular responses towards enhancing chronic wound healing, especially to bridge the
persistent inflammatory phase associated with most chronic wounds which consequently delay the healing process.

**Methods:** This systematic review aimed to identify devices that utilize electrical stimulation to enhance chronic wound healing. This study followed the Preferred Reporting Item for Systematic Review and Meta-Analysis (PRISMA) checklist. A literature search was conducted on studies published from 2000 to 2023 using Google Scholar, PubMed and ScienceDirect with the following keywords: wearable devices used for wound healing, wearable technologies used in wound healing, bioelectronic devices for wound healing, electroceutical devices used for wound healing. Inclusion criteria for published articles considered are specific to electroceutical devices that harness electrical stimulation for chronic wound healing. Exclusion criteria used to screen articles include wearable devices for monitoring body physiological and biochemical markers, devices delivering medication for infection control, devices using other wound healing modalities without electro-stimulation, and wearable electroceutical devices for acute wound healing. Titles and abstracts were screened to avoid duplicity and included those only with clinical data relevant to this review.

**Results:** A literature search revealed a total of 2235 related articles that mention the use of electrical stimulation. Out of these, 29 utilize electrical stimulation to enhance wound healing. Present wearable technologies support the possibility of using electrical stimulation as a concurrent or supplementary therapy in the management of chronic wound healing by harnessing the body’s endogenous electric field generated by ions. However, there is no standardized protocol that compares these various modalities to point out the ideal technology needed for translation into clinical use.

**Conclusion:** Wearable devices utilize various electro-stimulation patterns which include direct, alternating, and pulsed electric currents to stimulate cells, endogenous electric fields, angiogenesis, and growth factors for accelerated healing. The ideal device design remains an active research field for biomedical engineers. The need for soft-flexible devices in clinically-relevant form factors with occlusive and transparent wound dressings are necessary for such technologies to serve as adjuvant or primary treatment modalities for chronic wound healing.

**P75 | The Effect of Regenerative Debridement Therapy on Wound Bed Preparation with Respect to pH Modulation and Normalization**

Julian Bejarano³, Jeffrey M. Lawrence², Steven J. Kavros¹

¹Vascular Surgery Associates, Minneapolis, MN; ²Viroqua Center for Orthopedic Surgery, Viroqua, WI; ³Clyra Medical Technologies, Westminster, CA

**Introduction:** This study sought to evaluate the evidence of a novel topical, non-biologic technology employed for wound bed preparation with respect to pH modulation and stabilization. Regenerative Debridement Therapy (RDT) is a topical liquid agent for healthcare practitioners in the debridement of wounds, burns, and surgical site infections. It removes necrotic tissue, destroys biofilm upon contact, and reduces proinflammatory markers that become unbalanced in chronic wounds.

The purpose of the study is to examine the effect of RDT on pH in the wound bed of chronic DFUs and VLUs.

**Methods and Materials:** Adult patients with chronic DFUs and VLUs were considered eligible. Primary endpoint was pH collection at baseline, directly post sharp debridement, 30-60 seconds after sharp debridement, day 1, day 7, and day 14. 28 patients in the RDT group and 5 controls in each the DFU and VLU groups. Wound volumes were measured at each visit. SOC for DFU was hydrogel dressing and offloading. VLU was an alginate dressing and compression. All DFUs were planter Wagner grade 2 and VLUs were full thickness dermal wounds of the gator region.

**Results:**

<table>
<thead>
<tr>
<th>Table 1</th>
<th>DFU patients</th>
</tr>
</thead>
<tbody>
<tr>
<td>Table 2</td>
<td>VLU patients</td>
</tr>
<tr>
<td>Table 3</td>
<td>Percentage Volume Reduction – All patients</td>
</tr>
<tr>
<td>Table 4</td>
<td>Percentage Volume Reduction – DFUs</td>
</tr>
<tr>
<td>Table 5</td>
<td>Percentage Volume Reduction – VLUs</td>
</tr>
</tbody>
</table>

(all data is reviewed in the poster, as there are limitation due to abstract format)

**Conclusion:** There is a marked, extended reduction in wound bed pH with the use of Regenerative Debridement Therapy. The RDT allows the pH to maintain physiologic normal dermal pH for 7 days and further. This is with one, 30-60 second application after sharp debridement. There is also accelerated wound closure as seen with PVR grafts. Further studies are warranted.

**P76 | Wound Care in End-Stage Dermatomyositis: A Case Report**

James Bassett¹, Warren Back¹, Richard Simman²

¹University of Toledo College of Medicine, Toledo, OH; ²Department of Plastic Surgery, ProMedica Toledo Hospital, Toledo, OH

**Background:** Dermatomyositis (DM) is a rare inflammatory condition of the skin and muscle with a variety of clinical findings including characteristic skin presentations, calcium deposits, and concomitant cancer diagnoses. We share the case of a patient utilizing glucocorticoid treatment for end-stage DM and discuss the influence of disease and treatment that complicate wound management.

**Case Presentation:** A 60-year-old female presented to the wound clinic for slow healing hip and lower extremity wounds with signs of infection on both hip wounds despite negative cultures. She also exhibited extensive extremity and abdominal calcifications secondary to end-stage dermatomyositis and chronic daily glucocorticoid use of 7 years. She has a history of other immunosuppressive medications such as azathioprine, methotrexate, and infliximab injections in addition to history of previous surgical debridement. Despite meeting clinical criteria for DM, she had normal JO-1, RNA Polymerase III IgG, and
PM-Scl antibody levels found on testing. Multiple wounds were documented, with terribly slow healing courses, calcium deposits were drained in clinic, and finally surgical debridement was successful.

**Discussion:** Dermatomyositis presents numerous challenges in management, particularly in advanced stages of the disease. This case highlights a notable manifestation of slow wound healing, emphasizing the complexities involved in the treatment approach. Evidence of vasculopathy in DM has been integral to this process leading to compromised blood flow. More specifically, DM patients often have decreased capillary network density. Changes on these fronts affect the proliferation and remodeling phases of healing in addition to the inflammatory process that is inherently dysregulated in DM.

In managing this end-stage dermatomyositis case, conventional therapeutic approaches involving corticosteroids were implemented and continue to be utilized with this patient today, the current standard of care. Biologics such as monoclonal antibodies (rituximab, infliximab) also used have shown to be well tolerated in patients with dermatomyositis and polymyositis. Methotrexate, has also shown some efficacy. Azathioprine well targets the muscle involvement of DM, but there is limited literature suggesting efficacy addressing the cutaneous aspects of the disease.

**Conclusion:** In summary, this case report highlights the intricate challenges associated with managing end-stage dermatomyositis, notably emphasizing the complication of slow wound healing. Despite the efficacy of conventional treatments such as corticosteroids and adjunctive therapies like biologics and methotrexate, the persistent issue of impaired wound healing raises broader systemic implications of the disease.

A deeper understanding of the intricate vascular mechanisms could elucidate more targeted interventions.

**P77 | Establishment of a Protocol For Multicolor Flow Cytometry Of Porcine Skin Cells**

Shannon M. Clayton, Hsin-ya Yang, Cynthia Recendez, Kan Zhu, Guillermo Villa-Martinez, Anthony Gallegos, Min Zhao, Rivkah Isseroff, Athena Soulika

*Dermatology, UC Davis, Sacramento, CA*

Due to their similarity to human skin, pigs are increasingly used to study wound healing. Both pig and human skin have firm attachment, sparse hair coat, thick epidermis and dermis, no *panniculus carnosus*, and heal by re-epithelialization with minimal contraction.

Flow cytometry is a robust method to analyze the inflammatory milieu at the wound site. Although efforts have been made to optimize porcine skin digestion for flow cytometric analysis, it is currently not a common practice and there are no widely used protocols.

Our lab optimized a method to isolate single cells from porcine skin following excision injury using an enzymatic digestion that yield consistently robust single cell isolation with high viability. We also optimized reliable antibody panels, for the identification of granulocytes (CD45+2B2-SLADR+, CD11b+), monocytes subsets (CD45+2B2-SLADR-CD14+CD16+/−), as well as M1 inflammatory (CD45+2B2-SLADR+CD80+) and M2 reparative (CD45+2B2-SLADR+CD163+) macrophages. Development of a reliable method to isolate single cells from porcine skin opens the door for studies to assess the cellular changes during porcine wound healing.

**P78 | Understanding Barriers To Wound Care Access And Chronic Wound Management For Veterans**

Kelly Frasier1, Elliott Mitnik2

1*Internal Medicine, Nuvance Health, Wappingers Falls, NY; 2Castle Point VA, Wappingers Falls, NY*

Veterans encounter formidable barriers in accessing essential wound care and managing chronic wounds, stemming from a multifaceted interaction of systemic, geographic, and psychological factors. Geographical disparities pose a significant challenge, as specialized wound care facilities are often concentrated in urban areas, leaving veterans in remote or rural locations with limited access. Veterans are also at increased risk of experiencing hurdles such as limited access to specialized wound care facilities, fragmented healthcare coordination, and insufficient awareness among healthcare providers regarding the distinctive needs of veterans. Mental health issues prevalent among veterans, including post-traumatic stress disorder (PTSD) and depression, contribute to the complexity by impeding proactive engagement in self-care and exacerbating chronic wounds. Additionally, a lack of comprehensive training amongst healthcare providers in veteran-specific wound care further hinders effective treatment. These multifaceted barriers underscore the need for targeted interventions that address geographic disparities, streamline healthcare transitions, enhance mental health support, and provide specialized training for healthcare professionals. This review underscores the importance of furthering interventions to enhance accessibility, improve healthcare coordination, and increase awareness among healthcare professionals. Addressing these barriers is essential to ensure that veterans receive timely and effective wound care, promoting overall well-being, health, and quality of life.

**P79 | Case Study: Avoiding Hospitalization for a Limb-Threatening Diabetic Foot Infection with a Novel, Oral, Broad-spectrum, Anti-MRSA Tetracycline Antibiotic**

Alexandria Armstrong

*Orthopaedics, University of Texas Health Science Center San Antonio, San Antonio, TX*

**Purpose:** A tragic complication of diabetes is limb threatening soft tissue and bone infections of the lower extremities. Initial antibiotic choice steers the extent of limb preservation, avoidance of hospitalizations, and other sequelae. We present a patient successfully treated with an oral broad-spectrum tetracycline for a complicated diabetic foot infection, on an out-patient basis.
Methods: The treatment course of a patient with a severe foot and limb-threatening soft tissue and bone infection secondary to a diabetic foot ulceration was captured. Based on the clinical presentation of the diabetic foot infection, an oral broad-spectrum tetracycline was selected. The local signs and symptoms of a severe, complicated soft tissue infection were routinely assessed during the course of oral treatment with omadacycline. We surveilled edema, erythema, tissue and bone viability, deterioration of the wound, potential abscess formation and reported pain levels. The patient was evaluated until the successful resolution of her lower extremity infection.

Results: The patient underwent a ten-day course of oral therapy that was undertaken on an outpatient basis. She was strongly cautioned to rest and elevate the affected limb. She began with a loading dose of 450mg, then transitioned to a daily dose of 150mg orally for a total of ten days. The patient reported compliance with prescribed regimen. The infection site was scrutinized for signs of worsening, specifically for abscess formation or deterioration of wound margins. She did not need significant surgical intervention, in particular no resection of infected bone or amputations were performed. The patient’s site of infection demonstrated a consistent reduction in edema, erythema, and pain. Critically, the patient did not require hospitalization, major amputation or a PICC line.

Conclusion: Antibiotic empiric therapy for complicated, acute bacterial skin and skin structure infections is frequently too broad. Practitioners responsible for the antibiotic stewardship must become more mindful of the novel, safer antibiotic options that exist. There are newer, effective agents that reduce antibiotic-related complications that are better tolerated by patients. Via the selection of a targeted antibiotic, our patient avoided the common sequelae of a potent multi-drug antibiotic cocktail, whilst convalescing at home, and without the life altering side effects.

P80 | Adjunctive Biofilm Management Thru The Mitigation Of Microvascular Hyperpermeability; A Theory

Matthew M. Melin², Gregory S. Schultz³, Monika Gloviczki⁴, Matthew Regulski⁵, Raymond Shields¹
¹Vascular Medicine, HBO, Mayo Clinic, Rochester, MN; ²Vascular Medicine, Wound Care, Mayo Clinic, Rochester, MN; ³OB GYN, Research, Wound, University Florida, Gainesville, FL; ⁴Vascular Medicine, Mayo Clinic, Rochester, MN; ⁵Podiatric surgery, Ocean County Foot and Ankle, Toms River, NJ

Biofilms are implicated in delayed healing. The 2017 Global Consensus Panel publication established “step down, step up therapy” and “the need for strong initial combination treatment to rapidly and effectively reduce biofilm levels within wounds.” Wolcott et al. published a 2008 paper discussing a potential critical component to biofilm management thru the mitigation of microvascular hyperpermeability associated with wounds and the deprivation of biofilm nutrients.

The pro-inflammatory state occurs in 3D within soft tissues, including the posterior aspect of the wound, with associated microvascular and endothelial dysfunction and “glycocalyx shedding” (loss into the surrounding tissues of complex sugar components such as glycoproteins, proteoglycans, albumin, hyaluronic acid, etc). Shedding results in endothelial cell dysfunction (loss of mechanotransduction), diffuse microvascular hyperpermeability, resulting in loss of nitric oxide production, decreased inflammatory marker quenching, increased endothelial gap junctions. Dermal lymphatic stasis in peri-wound margins contributes to enhanced periwound inflammation. Endothelial glycocalyx and microvascular hyperpermeability potentially contribute to highly nutritious exudate from surrounding capillaries, enhancing biofilm sustainability.

Dysfunction of endothelial cells and shedding of the endothelial glycocalyx is well recognized to contribute to pathological conditions including diabetes, venous ulcers, atherosclerosis, sepsis, trauma. Could strategies to enhance glycocalyx preservation and/or restoration that improve endothelial cell function with decreased microvascular hyperpermeability enhance biofilm management by removing potential nutritional source to wound beds? The lowering of inflammatory markers, cytokines and associated edema reduction has been demonstrated to enhance healing of venous ulcerations with a variety of venotonics, including micronized purified flavonoid fraction, rutin-sides and sulodexide. L-Arginine has been noted to reduce edema in a rabbit reperfusion model.

The opportunity to improve outcomes may require a strategy that emphasizes an external biofilm and an “internal” approach that decreases microvascular hyperpermeability and edema, manages the associated inflammation, improves endothelial function, improves microvascular arterial perfusion, oxygen delivery, and dermal lymphatic function, while decreasing the “nutrient” source to the posterior aspect of wounds; an “outside/inside” approach to biofilm management that compliments the consensus guideline “step-down/step-up” biofilm therapeutic strategy. This remains a hypothesis, further benchtop to animal to clinical evaluation is necessary.

P81 | Maximizing Wound Care Treatment Post Discharge In Patients Affected By Homelessness

Kelly Frasier
Internal Medicine, Nuvance Health, Wappingers Falls, NY

Patients who are homeless regularly must overcome tremendous barriers to obtain health care post discharge from hospitals, surgeries, emergency departments, and urgent care clinics. Lack of health insurance and financial hardship are commonly experienced by many people in the United States living below the poverty line. Often, basic needs such as food and shelter outweigh obtaining proper healthcare. An aspect of healthcare that frequently burdens individuals who are homeless is proper wound care. With many homeless individuals experiencing multiple health comorbidities leading to chronic wounds (diabetic ulcers, chronic ulcers, venous insufficiency, lack of properly
fitting shoes, needle injuries, injuries from the environment, mental illness, post-surgical incisions), it appears imperative that we must do a better job at implementing effective wound care strategies when working with this specific population. This review prompts a current analysis of what the standard for wound care is in our homeless population in addition to what means this population has to obtain proper materials and education for wound healing. We propose a call to action for emergency departments, free clinics, and shelters to offer additional education and supplies for chronic wounds seen in patients experiencing homelessness.

P82 | Optimizing Infection Prevention in MOHS Procedures: Efficacy of Intraincisional Antibiotic Prophylaxis and Future Research Directions

Kelly Frasier1, Abigail Beard2
1Internal Medicine, Nuvance Health, Wappingers Falls, NY; 2Ohio University Heritage College of Osteopathic Medicine, Dublin, OH

Recent advancements in Mohs micrographic surgery (MOHS) procedures have demonstrated a significant reduction in the risk of postoperative infections through the implementation of novel practices. Notably, studies indicate that the use of incisional antibiotics has proven effective in decreasing the rate of surgical site infections associated with skin cancer surgery. Recent findings suggest that intraincisional antibiotic prophylaxis may offer a more efficient and localized method of infection prevention in MOHS procedures. While these emerging practices exhibit promise in reducing infection risks, further research is warranted to delve into the optimal strategies and specific agents for intraincisional antibiotic prophylaxis. Additional studies should explore the ideal timing, dosage, and duration of intraincisional antibiotic administration to maximize efficacy while minimizing potential adverse effects. Comparative analyses between intraincisional and systemic antibiotic prophylaxis could provide valuable insights into the most effective approach for different patient populations and surgical scenarios. Moreover, investigating the potential development of antibiotic resistance and the long-term implications of intraincisional prophylaxis is crucial to ensuring the sustainability and safety of these practices. This poster addresses the most recent findings regarding intraincisional antibiotic prophylaxis and explains the need for why further research is essential to address questions related to dosage, timing, and potential resistance development. Such investigations will contribute to refining guidelines for infection prevention in skin cancer surgery, ultimately enhancing patient outcomes and the overall success of MOHS procedures.

P83 | Wound Healing Properties Of The Mediterranean Diet

Kelly Frasier1, Michelle Hook Sobotka2
1Internal Medicine, Nuvance Health, Wappingers Falls, NY; 2Midwestern University Arizona College of Osteopathic Medicine, Glendale, AZ

The Mediterranean diet has proven itself effective in acute and chronic wound healing. A Mediterranean diet includes whole grains, vegetables, fruits, fish, extra virgin olive oil, red wine, and legumes. Foods studied within this diet contain high levels of antioxidants and anti-inflammatory compounds. The diversity of foods and numerous nutritional benefits maximizes wound healing with a variety of protective substances. The Mediterranean diet has high concentrations of polyphenols, carotenoids, vitamins, and flavonoids. Additionally, foods found within the Mediterranean diet are high in protein, zinc, vitamin A, and vitamin C that specifically aid in wound healing and the body’s defenses against infection. A low sodium Mediterranean diet has also been found to strengthen the activation of macrophages to increase the tissue inflammation process and promote wound healing. The consumption of extra virgin olive oil has been found to specifically lower the incidence of dermatological diseases. Specifically, extra virgin olive oil also plays an important role in increased platelet function thus having a direct effect on wound healing and decreased inflammation. Our review addresses how a Mediterranean diet aids with acute and chronic wound healing. The impact of nutrition on wound healing from a Mediterranean diet allows for development of a nutritional approach to minimize incidence of acute or chronic, non-healing wounds via dietary changes.

P84 | In Vivo Assessment Of Healing Efficacy Of Alkanna Tinctoria Water Extract On Full Thickness Burn Wounds

Ghayda Alzubaidy1, Shahad Bamulfilh3, Amal Almostady3, Faten Filimban1, Turki Zughaibi2, Suzan Alharbi1
1Biological Sciences, King Abdulaziz University, Jeddah, Outside of US & Canada, Saudi Arabia; 2Medical Laboratory Sciences, King Abdulaziz University, Jeddah, Outside of US & Canada, Saudi Arabia; 3King Fahad Medical Research Center, King Abdulaziz University, Jeddah, Outside of US & Canada, Saudi Arabia

Alkanna tinctoria is one of the plants used in folk medicine for many purposes, including wound healing. This study aims to evaluate the effect of A. tinctoria water extract on burn wound healing. 18 SWR mice were subjected to full-thickness burn in the dorsal area and were treated with different concentrations of water extract dissolved in beeswax and olive oil. The study includes six groups: negative (not treated), positive (1% silver sulfadiazine), vehicle (beeswax and olive oil), and 5%, 10%, and 15% water extract ointments, each having 3 mice in three independent experiments. Mice were treated and images were taken every two days for 14 days and skin and liver samples were collected at day 14. Images of the wounds were used for wound healing percentage during (0, 3, 6, 9, 12, and 14 days) post-burn using ImageJ software. Skin samples were used for histological measurement of epithelialization, and liver samples were used for hepatotoxicity examination. The wound healing percentage was higher in wounds treated with 5% water extract ointment compared to other groups, including the controls. Histological analysis revealed that 5% ointment has the best epithelization percentage and less
separation due to edema. Hepatotoxicity markers were not detected in all liver samples. In conclusion, the ointment prepared with 5% water extract showed promising therapeutic candidates for burn wound healing.

P85  |  Application Of Hydrogel Cellulose Nanofiber-Based Combination Scaffolds In Cutaneous Wound Healing

Judson V. Edwards², Michael Easson², Jacobs Jordan², Nicolette Prevost², Katie Hamel¹, Emma Rogers¹, Jordan Robinson¹, Haley Lassiter¹, Trivia Frazier¹, Cecilia Sanchez¹

¹Obatala Sciences, New Orleans, LA; ²ARS Southern Regional Research Center, U.S. Department of Agriculture, New Orleans, LA

**Background:** Cellulose nanofibers (CNF) are a fibrous form of elongated nanocellulose that has been the focus of widespread interest for wound healing and tissue engineering. CNF is non-immunogenic and biocompatible due in part to its propensity for low protein absorption. Thus, CNF with its high specific surface area and aqueous gelation properties is a biologically compliant scaffold for potential wound healing applications. We hypothesized that CNF/human-derived hydrogels could maintain structural, healing, and antibacterial properties to promote healing in minor to severely damaged skin e.g. primary wound healing treatment in burn injuries and chronic wounds. The purpose of the study was to develop proof of principle evidence demonstrating the feasibility of cotton and wood-derived CNF scaffolds/human-derived hydrogel combinations (NHC) for cutaneous wound healing applications.

**Methods:** The study involved the biophysical characterization, biocompatibility, and wound healing capacity of four mass ratio combinations for each of three forms of CNF. For characterization, rheology studies, evaluation of gelation time, protein release studies, proteomics, and microstructure analysis were performed with SEM. The biocompatibility of the nanocellulose/hydrogel combinations was assessed with the proliferative and adipogenic differentiation capacity of human adipose-derived stromal/stem cells (ASCs) and/or human dermal fibroblast cells (DFCs). Finally, wound healing studies were performed using ASCs and DFCs.

**Results:** CNFs provide support to human-derived material hydrogels. Rheology results demonstrated that nanocellulose responds similarly in combination with human-derived hydrogels. The nanomaterials increase viscosity and hydrogel stiffness in combination with human derived hydrogels. Biocompatibility, promotion of dermal fibroblasts proliferation and ASCs differentiation were assessed. ASCs cultured on a mixture of nanocellulose/hydrogel combinations exhibited increased metabolic activity from days 1-7 and intracellular lipid deposition after adipogenic differentiation. Notably, up to twenty five percent by mass of nanocellulose in the NHCs provides hydrogel support and porosity for cell proliferation. A comparison of three forms of CNF revealed a difference in in vitro wound healing profiles with one form of CNF providing notably enhanced proliferative profiles in the presence of NHC over controls. Proteomics revealed the presence of plant-derived protein, suggesting oversight guidance and activity profiles for CNF preparation.

**Conclusions:** Hydrogel/cotton and wound-based nanocellulose combination scaffolds display a unique biophysical and biochemical profile that supports human ASC and DFC proliferation, differentiation, and wound healing capacity.

P86  |  Systemic Inflammation, Wound-Related Symptoms And Biofilm In Older Adults With Chronic Venous Leg Ulcers (CVLU)

Jung Lyun Kim¹, Joyce Stechmiller², Michael T. Weaver², Garth James³, Phill Stewart³, Debra E. Lyon²

¹Chungnam National University College of Nursing, Daejeon, Korea (the Republic of); ²University of Florida College of Nursing, Gainesville, FL; ³Montana state University, Bozeman, MT

**Background:** About 8.2 million people are suffering from chronic wounds, and the treatment costs of chronic wounds ranged from $28.1 to 96.8 billion in 2014 in US. Since venous leg ulcers (VLUs) tend to be chronic due to their high susceptibility to infection and high recurrence rate, they account for the majority of chronic wounds. Patients with venous leg ulcers suffer from diverse symptoms, including pain, fatigue, depression, swelling and exudate, and most patients with VLUs who have delayed healing experience significant symptoms. Biofilm is recognized as an important component of wound non-healing and it is believed that the formation of biofilm delays wound healing. Therefore, by examining wound-related symptoms corresponding to biofilm and inflammatory markers, such as CRP, during the course of wound treatments, clinicians may predict wound healing trajectories. The purpose of this observational prospective study was to 1) characterize the wound-related symptoms (fatigue, pain, exudate, itching, and edema or swelling) and wound related factors (wound area, the presence of biofilm, total bacteria, the level of serum CRP), and 2) explore associations between biofilm and levels of systemic inflammation and the severity of wound-related symptoms in individuals with chronic venous leg ulcers (CVLU) over 8 weeks of wound treatment.

**Methods:** A total of 117 subjects who received weekly sharp debridement at a wound clinic were enrolled. We collected clinical data every two weeks during the 8 weeks of the study period. Associations among variables were estimated using a Bayesian approach applied to general linear mixed models.

**Results:** Based on Bayes Factor (BF) value, there was moderate evidence of a direct association between biofilm presence and levels of C-reactive protein (CRP) (BF 4.3) and moderate evidence of indirect associations between biofilm and wound-related symptoms: pain and exudate (BF 5.12, 8.49 respectively). There was extremely strong evidence for the association of biofilm with mean total bacteria.

**Conclusion:** This study is the first to examine associations among biofilm, inflammatory response, wound-related symptoms, and wound healing in clinical settings. Wound-related symptoms and the level of systemic CRP were associated with biofilm among patients who were
receiving weekly sharp debridement. Symptom severity associated with CVLU requires clinical assessment and management. Symptom severity and level of systemic CRP may be biobehavioral markers for predicting wound healing trajectories.

**P87 | Association Of Tryptophan/Kynurenine Metabolites With Healing In Chronic Venous Leg Ulcers**

Jung Lyun Kim¹, Joyce Stechmiller², Michael T. Weaver², Debra E. Lyon², Timothy Garrett³, Fan Yi⁴, Debra Lynch Kelly²
¹Chungnam National University College of Nursing, Daejeon, Korea (the Republic of); ²University of Florida College of Nursing, Gainesville, FL; ³University of Florida College of Medicine, Gainesville, FL; ⁴University of Idaho, Moscow, ID

**Background:** Chronic wound healing is a complex process that is still not well understood. The tryptophan (TRP)-L-Kynurenine (KYN) pathway increased scrutiny in wound healing. Indoleamine 2,3-dioxygenase (IDO) which mediates Tryptophan catabolism is highly active during pathological conditions including chronic wounds. We utilized metabolomics to investigate the biomarker involved in the TRP-L- KYN pathway in chronic venous leg ulcers (CVLUs) healing. The study aims to apply metabolomics to elucidate the TRP-L- KYN pathway associated with CVLUs wound healing.

**Methods:** The study was a longitudinal design, involving 60 serum samples collected from 30 older adult patients with CVLUs, who received weekly sharp debridement at a university wound clinic. The serum samples were collected at baseline, week 4, and week 8 (or at the time of wound closure). Liquid chromatography-mass spectrometry (LC-MS) metabolomics was used to analyze targeted metabolites. A Bayesian approach was employed to examine robust correlations between changes in metabolite values and linear healing slope.

**Results:** The mean age was 71.13 (±9.46); The healing group (n=23) demonstrated higher levels of mean TRP at baseline and overtime compared to the non-healing group. There was moderate support for a negative association between kynurenic acid and steeper healing slopes (r = -0.36, Crl = -0.62, -0.06).

**Conclusion:** Gaining a better understanding of the associations between the TRP-L- KYN pathway and the healing of CVLUs could help to clarify the links of inflammation with the rate and success of wound healing. Biomarker development focused on the TRP-L- KYN pathway could be pursued, if the associations are further supported by focused research studies.

**P88 | Psychometric Validation of the Korean Pressure Ulcer Knowledge Assessment Tool**

Jung Y. Kim
Wound care nursing department, seoul national university bundang hospital, Gyeonggido, Korea (the Republic of)

Pressure ulcers are a major issue in contemporary healthcare, with prevalence and incidence rates of 12.8 and 5.4% respectively based on the 2008-2018 data from a systematic review in 2020. The implementation of pressure ulcers (PUs) prevention is essential for all patients however only two-thirds of Korean nurses are reported to be performing PU prevention tasks, such as risk assessment. Nurse with greater knowledge of PU prevention would be expected to perform it more often than those with less knowledge. The purpose of this study was to evaluate the psychometric properties, including content validity, validity of multiple choice items, and the reliability of the Korean version of the Pressure Ulcer Knowledge Assessment Tool (K-PUKAT 2.0), using classical test theory (CTT) and item response theory (IRT). Linguistic validation process and factor analysis were conducted among wound care nurses, staff nurses and nursing students. Items were analysed according to the CTT and IRT using a two-parameter logistic model. Intraclass correlation coefficients were used to examine reliability.

A total of 378 wound care nurses, staff nurses and nursing students participated in this study. While most items showed moderate difficulty based on the CTT, difficulty index values based on the IRT were more broadly distributed (low: 5 items; moderate: 9 items; high: 1 item). The intraclass correlation coefficient for K-PUKAT 2.0 was 0.72.

The K-PUKAT 2.0 demonstrated concise and good psychometric properties. Based on the results of this study, repetitive use of K-PUKAT 2.0 will not only help in distinguishing whether an individual has sufficient clinical knowledge, but will also play a key role in supporting learning.

**P89 | Fibroblasts Show Differential Expression Of Interleukins Across Monolayers Injured In Vitro**

Erin Aucoin, Kelsey Wilson, Emma Kennard, William Lindblad
Pharmaceutical Sciences, Husson University, Bangor, ME

Expression of pro-inflammatory cytokines by human dermal fibroblasts occurs when the cells are activated by cellular injury. We have reported previously, using an in vitro wound model, that within 8-12 hours of wounding a fibroblast monolayer, cells express and secrete a number of cytokines including interleukin (IL) -1, 6 and 8. We had hypothesized that this expression arose from cells immediately adjacent to the area of wounding, however we now report that cells at several cell distances from the wound grid express these cytokines suggesting that a compound released from injured cells may trigger this cell expression.

Non-transformed human dermal fibroblasts (GM23973 and GM1872, Coriell Institute) were obtained from the NIGMS cell bank and cultured under standard conditions. The cells were seeded onto acid-stripped glass coverslips grown in 6-well plates using DMEM medium containing 10% FBS and penicillin/streptomycin. Upon confluence, the coverslips had cells scraped in a grid pattern and IL-8 localized by immunofluorescence using FITC-labelled primary antibody.
(BD Bioscience) at various times post scraping. Total immunoreactive IL-8 secretion from confluent cell monolayers following scraping was also performed in large cell culture dishes by ELISA (Raybiotech) and Western blotting.

Findings of note were that cells immediately adjacent to the scraped area showed variable expression of IL-8 with many cells not showing any expression. In contrast, cells away from the scraped edge showed the brightest immunofluorescence. This suggested that cells at the immediate edge, which are either migrating or entering for cell division, may not also express pro-inflammatory genes. These cells may release soluble mediators to stimulate other cells in the wound area to express inflammatory mediators, or factors released from the scraped cells are able to act as paracrine mediators.

Based on cell injury in epithelial cells, we also examined the potential that uridine 5'-diphosphate (UDP) released from damaged fibroblasts could serve as a soluble mediator to activate the distant cells. Preliminary studies with LC/MSMS failed to detect significant levels of this nucleotide in culture media after scraping. Addition of exogenous UDP also failed to significantly enhance fibroblast monolayer expression of these ILs. Suggesting that in fibroblasts this compound is not involved with the injury response.

These findings suggest that fibroblast IL expression is dynamically regulated with cells triggered to move and/or proliferate showing less IL synthesis whereas cells that are not directly impacted by injury show significant expression. Understanding the biochemical triggers for this induced production of ILs may lead to pharmacologic approaches to reduce excess inflammation in problematic wounds.

**P90 | A Novel Bio-Inspired Skin Graft Adhesive**

Zachary Everett1, Britani Blackstone1, Dorothy Supp2, Heather Powell1

1 The Ohio State University, Mason, OH, OH; 2 Surgery, University of Cincinnati, Cincinnati, OH

Full-thickness burn injuries are commonly treated with autologous split-thickness skin grafts (STSGs). Different strategies may be used to hold STSGs in place including staples and vacuum dressings. To enhance fixation across the entire graft, fibrin glue may be sprayed onto the wound bed prior to graft placement. Though fibrin glue significantly improves graft adhesion to the wound bed, it can be expensive and has been reported to increase inflammation and impair epithelial cell migration.

The goal of this study was develop a new strategy for promoting adhesion of STSGs a to the wound bed. To achieve this, polydopamine nanoparticles (PDA-NPs) were produced by dissolving 0.5mg/mL dopamine hydrochloride-anhydrous (DHA) in water. The DHA solution was then titrated with 1N NaOH at a ratio of 3.333 mL NaOH/ml DHA solution. The resulting solution was aliquoted into micro-centrifuge tubes and centrifuged at 13,750rpm. The pellets were lyophilized for 18 hours then diluted in un-supplemented DMEM with bovine serum albumin (BSA) to the desired PDA-NP concentration. To examine adhesion strength as a function of PDA-NP concentration and type/duration of near infrared laser excitation, human surgical discard tissue was collected from paninciculectomies and STSGs harvested (0.008”). To create a “wound bed”, an additional piece of tissue was harvested below the STSG at a depth of 0.032”.

Dog-bone shaped samples were punched from STSG and the wound bed, and PDA-NP-BSA solution applied to the tab portion of the dog bone punches at a volume of 30µL/cm² and a PDA-NP concentration between 0-1000µg/cm². PDA-NPs were excited by exposure to near infrared (NIR) laser in different durations and exposure patterns. Samples were then secured into a uniaxial tensile tester and strained at 1mm/sec.

Initial testing with a PDA-NP at a concentration of 1000µg/cm² with a fine pattern NIR laser exposure (3x3 grid with ~1mm diameter spot size) significantly improved adhesion compared to wide NIR laser (1x2 grid with 1-3mm diameter spot size) and 0 µg/cm² controls. Subsequent testing showed that using 1000 µg/cm² PDA-NPs with 15 min incubation on the wound bed prior to NIR excitation resulted in an average adhesion force of 0.13N while using 500 µg/cm² with incubation yielded an average of 0.11N Biocompatibility assessed via MTT assay, showed no difference in no NIR exposure, no PDA-NPs controls and any PDA-NP concentration (0-1000 µg/cm²) or NIR exposure (fine or wide). Studies are ongoing examining the adhesion of human STSGs to full-thickness wounds in athymic mice compared to fibrin glue along with an examination of STSG integration and inflammation in this model.

The above results suggest that PDA-NP-BSA solutions can be used as a medical adhesive to secure STSGs to the wound bed and may provide improvements in associated inflammation compared to fibrin-based glues.

**P91 | Polydatin Attenuates Burn-Induced Vascular Hyperpermeability By Inhibiting Mitochondrial-Dependent Apoptotic Pathway**

Huining Bian, Chuanwei Sun, Hongmin Luo, Hanhua Li, Wen Lai

Division of Burns and Wound Repair surgery, Guangdong Provincial People’s Hospital, Guangzhou, Guangdong, China

**Backgrounds:** Polydatin (PD, 3, 4’, 5-Trihydroxystibene-3-beta-monogluicoside) is a monocristalline drug isolated from Polygonum Cuspidatum. It has obtain permission for phase II clinical trials from the Chinese Food and Drug Administration (Clinical Trials.gov identifier: 2006L00301), as well as from the American Food and Drug Administration (Clinical Trials.gov identifier: NCT 01780129). This study tests the hypothesis that polydatin attenuate vascular hyperpermeability after burn by inhibiting the activation of the intrinsic apoptotic pathway.

**Materials and Methods:** Scalp-induced edema model of rat ears was used to find out the effect of polydatin gel. Experimental rats which subjected to burn injury covering 30% of the total body surface area were used to explore its mechanism. PD, cyclosporine A (CsA), or resveratrol (Res) was administered to the animal model after burn injury.
The rats were injected with fluorescein isothiocyanate albumin (50 mg/kg), and changes in the integrated optical intensity of the postcapillary venules were determined by intravital microscopy. The permeability of mesenteric venules was assessed. Expression of cytochrome C and Smac in cytosolic fraction, as well as expression of Bcl-2 and Bax in mitochondrial fraction, were analyzed by Western Blotting. Caspase-3 activity was detected with Caspase-3/CPP32 Fluorometric Assay Kit.

Results: The permeability coefficient of the burn skin venules in the burn+NS group was higher than that in the sham burn group (P< 0.01). By the video micro scale, afferent swelling was obvious in rats immediately after burn, and no blood circulation could be detected. The wound edema reduced and the blood flow began recovery after 6 hours after PD administrating. Treated with CsA, Res, or PD resulted in a reduction in the FITC-BSA extravasation. The results of Western Blotting indicated that the levels of cytochrome c and Smac in the cytosol of the mesenteric vasculature in the sham burn group were significantly lower than those in the burn+NS group at 6 h after burn. Treatment with CsA, Res, or PD reduced the cytosolic release of cytochrome c and Smac. This reduction was more significant in the burn+Res and burn+PD groups (both, P< 0.01) than in the burn+NS group. Burn injury resulted in the upregulation of Bax proteins and downregulation of Bcl-2 in the rat mesenteric microvasculature. Treatment with CsA, Res, or PD could attenuate these changes to some extent. The level of caspase-3 activity in the mesenteric microvasculature showed that the level was increase in the burn+NS group, the burn+Res and burn+PD groups showed significantly lower levels (both P< 0.01) than the burn+NS group after treatment.

Conclusion: PD markedly attenuated burn-induced local and systemic hyperpermeability which could be detected by the activation of endogenous apoptotic signaling pathway.

P92  |  Plastic Surgery Techniques For Medial Forefoot Wound Closure

Nicholas Blasingame, Connor Krolikowski, Collin Pehde  
Orthopaedics, UT Health San Antonio, San Antonio, TX

Background: All too frequently, non-viable, infected, or traumatized extremities are amputated leaving an open wound. Diabetics with a foot wound are more likely to be depressed, anxious and have a 2.5-times increase in risk of death compared to diabetics without a foot wound. More than half of these wounds become infected. The longer a wound is open increases odds of infection, thus making the goal in treatment for an open diabetic foot wound: a plantigrade foot. From blood glucose control to off-loading/bracing, which contribute to the wound, need to be addressed.

Purpose: This case series presents plastic surgery techniques for medial forefoot soft tissue reconstruction in order to achieve closure for chronic and acute wounds following amputation.

Methods: Cases of two patients with medial forefoot wounds following amputations from diabetic foot infections underwent reconstructive procedures to achieve closure. Complete healing was assessed by the time to full epithelialization of surgical sites.

Procedures: Satterfield-Jolly random rotational fasciocutaneous flap, toe fillet flap, full thickness sinus tarsi graft.

Results: Random rotational flap with toe fillet flap achieved full healing by 3 months. Sinus tarsi full thickness graft had partial loss of graft, progressed to heal secondarily.

Conclusions: When faced with soft tissue deficits following amputation, surgeons should be well trained in various options for closure. To have successful soft-tissue reconstruction all systematic components from blood glucose control to off-loading/bracing, which contribute to the wound, need to be addressed.

P93  |  NEWT Plasma Inhibit Differentiation Of Mouse Fibroblasts Into Myofibroblasts And Contribute To Wound Regeneration

Tatsuyuki Ishii1, Ikkei Takashimizu2, Yusuke Yoshioka4, Chikafumi Chiba3, Kazuo Kishi1
1plastic and reconstructive surgery, Keio University, Shinjuku-ku, Tokyo, Japan; 2plastic and reconstructive surgery, Shinshu University, Matsumoto, Nagano, Japan; 3Faculty of Life and Environmental Sciences, Tsukuba University, Tsukuba, Japan; 4Department of Molecular and Cellular Medicine, Tokyo Medical University, Shinjuku-ku, Tokyo, Japan

While scar healing by fibrosis is a wound healing strategy that we mammals are equipped with, it is also the cause of many intractable diseases such as keloids and pulmonary fibrosis. Although numerous studies have been conducted to inhibit fibrosis, the inflammatory response that leads to fibrosis is also required for beneficial repair processes, so targeting it for disease treatment is still expected to be challenging. To identify fibrosis-suppressed regenerative therapeutic strategies, we focused on the special regenerative potential of newts, which belong to the salamander family of tailed amphibians. Many studies have shown that newts maintain a strong regenerative capacity throughout life without fibrosis even after reaching adulthood beyond metamorphosis. Considering that even amphibians with strong regenerative abilities lose or decline their regenerative abilities as they grow, it is likely that newts have an unknown regenerative factor. In this study, we report our findings that newt plasma may confer fibrosis-inhibitory properties on mouse fibroblasts across species. First, to determine the effect of newt plasma on mouse fibroblasts, we examined cell viability after the addition of newt plasma. To avoid variation due to individual differences, whole blood was collected from each of five blastema. After isolating plasma from the whole blood samples, cell viability at 72 hours was evaluated. The results showed that the administration of newt plasma within the range of 0.1% to 1% had no lethal effect on mouse fibroblasts. Next, we tested the inhibitory effect of the addition of newt plasma on fibrosis by using TGFβ1 to induce fibroblast differentiation into myofibroblasts in an assay. Fluorescent immunostaining using F-actin and αSMA as markers showed that the expression of both was attenuated by the
addition of newt plasma. Quantitative evaluation using Western blotting confirmed that the addition of newt plasma reduced the amount of αSMA to the same level as the control group. This suggests that the addition of newt plasma may have inhibited the induction of differentiation into myofibroblasts. To further identify the location of fibrosis inhibitory factors, we focused on extracellular vesicles (EVs) and isolated EVs from newt plasma by ultracentrifugation. We performed the same experiment with the isolated EVs as with plasma addition, and found that the same effect was obtained. This suggests that newt plasma has the ability to suppress fibrosis across animal species, and that it is highly likely that plasma EVs contain factors that can suppress fibrosis. This research may offer new possibilities for regenerative medicine.

P94 | A New Treatment For Long-Term Diabetic Wounds

Sufan Chien1, Harshini Sarojini1, Samson Chien2, Sufan Chien2
1Surgery, University of Louisville, Floyds Knobs, IN; 2Chemistry, Noveratech, LLC, Louisville, KY

Background: In medicine, the treatment of diabetic wounds is one of the most difficult to get good results. Despite thousands of wound dressings developed in the past century, none has shown any good effect to enhance the healing process, including the only FDA-approved prescription growth factor, Regranex.

Multiple factors contribute to nonhealing diabetic wounds, and many of the pathways involved are not entirely clear. Surgeons have long recognized that ischemic tissues heal poorly and are easily infected. One direct effect of ischemia is a reduction of tissue high energy phosphate reserve.

Methods: We have developed an intracellular ATP delivery (ATP-vesicles) to enhance wound healing by providing direct high energy phosphate. When used in animal experiments, it has shown extremely rapid tissue regeneration—granulation tissue starts to appear very quickly after surgery. We hypothesized that tissue ischemia was one major contributing factor in diabetic wound healing, and if exogenous energy is provided, healing should be enhanced.

Twenty-five diabetic rabbits were used in this study. Diabetes was induced by alloxan (100 mg/kg) IV injection. After stable diabetes was established, the rabbits were kept for 3-12 months before wound study. In each rabbit, the left ear was made ischemic using vascular disruption or adding a silicone ring buried in the ear base to limit vessel and nerve regeneration. The right ear vessel and nerve supply was not disturbed as normal control. Four wounds (5 mm in diameter) were created on the ventral side of each ear, resulting in 100 ischemic wounds and 100 non-ischemic wounds. On each ear, the two wounds on one side were treated with ATP-vesicles while the other two wounds were treated with Regranex (50 for ATP-vesicles and 50 for Regranex).

Results: Many of the wounds treated by ATP-vesicles started to have new growth only 1 to 2 days after surgery while the wounds treated by Regranex did not have new growth before day 5. The wounds treated by ATP-vesicles were healed much faster than the wounds treated by Regranex. Histologic study shows that the use of ATP-vesicles increased tissue collagen production quickly and this was accompanied by very early and massive platelet trafficking followed by massive macrophage accumulation and rapid direct collagen production, resulting in much faster wound healing.

Conclusion: Our results show that using intracellular ATP delivery can enhance wound healing in long-term diabetes, and this is accompanied by rapid macrophage accumulation, in situ proliferation, and direct collagen production.

P95 | Topical, Fibonectin (FN)-Derived, cNP8 Peptide Speeds Burn Healing And Has Antimicrobial Activity

Richard A. Clark, Fubao Lin, Michael C. Musillo
NeoMatrix Therapeutics, Stony Brook, NY

Background: Previously (WHSS 2017), we presented a re-engineered FN-derived peptide that could resist neutrophil elastase (cNP8) and potentially speed burn healing intravenously (IV) or topically. Subsequently, we demonstrated that IV cNP8 speeds burn healing and decreases scarring (JID 140:1480-1483, 2020). Now we show that topical cNP8 speeds burn healing, decreases scarring, and is an antimicrobial peptide (AMP).

Methods: Our vertical injury progression porcine burn model (J Burn Care Res. 32:638-46, 2011) was used for these studies. Partial-thickness burns (2.5x2.5cm) were debrided with 1 (0.75mm) or 2 (1.5mm) dermatome passes at 24h post burn. Next, 0, 300, 600, or 900μM cNP8 was applied in 0.5ml PBS containing 1.2mg collagen. At 10d, 14d and 28d, biopsies were taken from each wound to determine re-epithelialization (re-epi), wound closure (WC), and scarring (SC), respectively, from 5μm sections of hematoxylin & eosin stained, formalin-fixed tissue. Antimicrobial activity of cNP8 against S. aureus and P. aeruginosa was performed by Situ Biosciences, Inc., an ISO-certified laboratory. Using an Agar Plate assay, we investigated whether human serum inhibits cNP8 killing of E. coli (K12).

Results: Outbred pigs typically show much variability among breeding cohorts. In our first cohort of 4 pigs, a 1-dermatome pass resulted in better burn healing compared to 2-dermatome passes. After 1-dermatome pass in burn wounds: 600μM cNP8 showed 90% WC and 100% median re-epi compared to 900μM cNP8 (10% WC and 74% median re-epi) or collagen gel control (0% WC and 32% re-epi). For SC, 600μM cNP8 showed 3mm median SC depth compared to 900μM cNP8 or collagen gel control (4mm median SC depth).

In 3 cohorts (12 pigs), after 1-dermatome pass of burns: 300μM cNP8 showed 50% WC and 99% median re-epi; 600μM cNP8 (63% WC and 100% median re-epi); and collagen gel control (70% WC and 73% re-epi). For SC, 300μM cNP8, 600μM cNP8 and collagen gel control showed 5mm median SC depth. The 900μM cNP8 dose was not tested after the first cohort.

Studies at Situ Biosciences demonstrated that serum-free 10μM cNP8 kills all P. aeruginosa and S. aureus within 24h, consistent with
antimicrobial peptide (AMP) activity. In our studies, we found that 50% and 75% serum inhibited cNP8 antimicrobial activity up to 50μM, but not at 100μM, 300μM, or 600μM. 100% serum alone kills all E. coli so the effect on cNP8 killing could not be determined. Preliminary data of wound fluid showed no inhibition of cNP8 killing of E. coli.

**Conclusion:** Since IV cNP8 dilates mucocutaneous microvasculature (100pM to 1μM; two papers), topical cNP8 promotes tissue cell survival and growth (10 - 100μM, multiple papers), and topical cNP8 may be a broad-spectrum antimicrobial that resists serum degradation, we posit that IV and topical cNP8 will be potent, synergistic therapies for burns and chronic wounds.

---

**P96 | The Promotion Of Critically Colonized Wound Healing By Cleansing With Soforo-Fine Bubbles**

Katsunori Kato5, Takeo Minematsu5, Chiihiro Takizawa2, Sanai Tomida2, Yoriko Kato3, Yuka Oda3, Yoshihiko Hirata3, Miyako Wakiizaka3, Yoko Hasagawa4, Kazuhiro Ogai4, Gojiro Nakagami2, Chizuko Konya5, Hiromi Sanada6

1Graduate School of Nursing, Ishikawa Prefectural Nursing University, Kahoku, Ishikawa, Japan; 2Department of Gerontological Nursing/Wound Care Management, The University of Tokyo, Hongo, Tokyo, Japan; 3Biochemical Laboratory, Saraya Co., Ltd., Kashiwabara, Osaka, Japan; 4Department of Bio-engineering Nursing, Ishikawa Prefectural Nursing University, Kahoku, Ishikawa, Japan; 5Department of Adult Nursing, Ishikawa Prefectural Nursing University, Kahoku, Ishikawa, Japan; 6Ishikawa Prefectural Nursing University, Kahoku, Ishikawa, Japan

Ultra-fine bubbles, measuring less than 1 μm in diameter, are stable and effective for cleansing various materials. Recently, a chemical method using sophorose lipids has been developed to generate ultra-fine bubbles, known as Soforo-fine bubbles (SFB). Previous reports have highlighted the efficient removal of *in vitro* biofilms by SFB. This study aims to demonstrate the practical utility of SFB in cleansing critically colonized wounds through two animal experiments. Two full-thickness wounds were created on the dorsal skin of a rat and cleansed daily with either SFB or control solutions. In Experiment 1, normal wounds were created to assess the safety of SFB. In Experiment 2, critically colonized wounds were prepared to demonstrate the effect of SFB on wound healing. In both experiments, the healing period and relative wound area were compared between the groups. This study was conducted with the approval of the Animal Experimentation Committee of the University of Tokyo. In Experiment 1, there was no difference in the healing period between groups (p = 0.178). On post-wounding days (PWDs) 9, 10, 12, and 14, the relative wound area was significantly smaller in the SFB group compared with the control group. In Experiment 2, the SFB group had a significantly shorter healing period (14.3 ± 0.50 days vs. 15.5 ± 0.58 days, p = 0.015) and a smaller wound area on PWDs 5, 6, 9, and 13 compared with the control group. These results indicate that cleansing with SFB has no negative effect on normal wound healing and, importantly, promotes healing in critically colonized wounds.

**P97 | Acute Wound Bleed and Novel Biodegradable Hemostatic Device**

Ilya Kleyn1, Daniel Beckles2

1Clinical R & D, NOIGEL LLC, New York, NY; 2Cardiothoracic surgeon, Baylor Scott & White Health, Round Rock, TX

**Background:** The leading cause of death on the battlefield, in surgical wards, and in other severe trauma situations is active bleeding. Current hemostatic devices form a powdery substance of non-degradable minimal-absorbable efficacy granules, which in turn irritate and delay wound healing, additionally, the exothermic effect of the granules can cause second-degree burns and at times it may lead to necrosis of the surrounding tissues.

With all these in mind, our researchers developed a powdered composition (substance). In our composition, the natural semisynthetic polymers break down into micro molecular elements, similar to glucose, and are eliminated from the body without a trace, without side effects or reaction to the host body.

**Methods & Results:** Resorption rate: In vitro studies Celox and our K1 as a study material weighing 1 g each placed in a measuring tube containing 5 ml of distilled water. Then it was placed in a thermostat with a constant temperature of 37 °C. The rate of biological degradation of hemostatic material was evaluated on days 1, 3, 7, and 14. The studied hemostatic material was dried. The difference in the mass of the hemostatic material before the experimental study and after its implementation was measured in percent and reflected the rate of resorption of the studied agent (HM). The maximum resorptive activity was observed with the K1 composition.

Sorption activity: In vitro studies Celox and our K1 as a study material weighing 1 g each put into distilled water. The degree of full saturation of the studied hemostatic material (HM) was determined visually, based on the changes in swelling. To assess the sorption activity of the studied materials, their hygroscopicity was determined. For a comprehensive assessment of the sorption properties of hemostatic materials, we used a sorption index (SI). The sorption rate of K1 was far superior to Celox.

Bleeding time based on compression effect: The experiment was carried out on 80 male Wistar rats. A median laparotomy was performed under anesthesia and modeled a standard injury of the liver and spleen. A hemostatic agent was applied in the wound area in the amount fitting to the size of the injury. Bleeding time was measured with a stopwatch.

The bleeding time from liver injury after applying K1 was decreased by 89.5-93.5% relative to the control group with similar injuries. The shortening time needed to stop bleeding from the experimental spleen injury was mainly observed with the K1 compound (p <0.001).

**Conclusions:** Our new generation of hemostatic substances with a binary effect can be used on the battlefield, in hospitals, and by
surgeons in life-threatening uncontrolled bleeding situations. It absorbs 70 times the amount of blood compared to existing hemostatic products and is easy to manufacture.

P98  Determination of Dipasol FC and Dipasol Compositions Impact On Tissue Regeneration

Ilya Kleyn1, Artur Martynov2, Boris Farber3, Daniel Beckles4
1Clinical R&D, NOIGEL LLC, New York, NY; 2Laboratory and clinical department of molecular immunopharmacology, I. Mechnikov Institute of Microbiology and Immunology of National Academy of Medical Sciences of Ukraine, Kharkiv, Ukraine; 3R&D Department, NOIGEL LLC, New York, NY; 4Cardiac Thoracic Surgery, Baylor Scott & White Health, Round Rock, TX

Background: Pluripotent stem cells are known for their role in rejuvenation and supporting a robust immune system. Pluripotent stem cells become scarce as the body ages. To overcome these challenges, our researchers identified two groups of compounds cAMP phosphodiesterase and histone deacetylase inhibitors which, when combined, significantly increased the yield of stem cells after genetic modification. Despite the need for genetic modification, the yield of stem cells increased substantially a thousand-fold.

Methods: To assess the wound-healing properties of different compounds, experiments were conducted on male Wistar white rats with skin wound. A total of 38 animals were previously anesthetized, and a skin area measuring 2 by 2 cm was cut out on the dorsal side of the body, behind the right shield bone. Using forceps, the skin was pulled back, resulting in a skin fragment of 2 cm in size and a cut depth of 2 mm, leading to an average wound area of 3-5 cm². The wound, characterized by polygonal shape, exhibited intense bleeding. Subsequently, two substances, Dipasol FC and Dipasol aerosol substances, were applied to the wound of groups 1 and 2 (each consisting of 10 rats). Group 3, also comprising 10 rats, was treated with "panthenol," while the wound of the control group (group 4, consisting of 8 animals) were left untreated. The application of these substances involved ensuring that the formed aerosols covered the entire wound surface and a small skin area around it. BF-6 glue was subsequently applied on top of the aerosol and left to dry. Following this, the animals were reintroduced to their cages. A parallel study was also conducted on guinea pigs.

Results: A planimetric study was conducted on days 3, 6, 9, 11, and 13 after the start of the experiment to evaluate reparative processes before complete wound healing. The assessment involved applying celluoid film to the wound, plotting wound contours on the film, and determining the wound surface area using graph paper. This methodology provided insights into the features of the wound healing process over time, allowing for a comprehensive analysis of the effectiveness of the tested substances in promoting skin regeneration.

Conclusions: In a rat model with stencil wound, the topical aerosol application of Dipasol accelerated skin regeneration by 2 times. A guinea pig study Dipasol aerosol application accelerated wound healing by 5 times. These findings suggest a potential breakthrough in regenerative medicine, paving the way for improved wound healing and possibly addressing the challenges associated with aging through pluripotent stem cell enhancement.

P99  Development Of Novel Micro-RNA/Cerium Oxide-Based Polymer Composites For Wound Healing Applications

Elayaraja Kolanthai1, Craig J. Neal1, Yifei Fu1, Roshna Cherugil1, Kenneth W. Liechty2, Sudipta Seal1
1Material Science and Engineering, University of Central Florida, Orlando, FL; 2Department of Surgery College of Medicine, The University of Arizona, Tucson, AZ

Background: Diabetic wounds represent significant challenges in healthcare, impacting over six million individuals in the United States. Compromised healing, resulting from diabetic condition, is exacerbated by the formation of biofilms. The progression of infection results in inflammation and reactive oxygen species (ROS), contributing to vascular damage and attenuated healing rates at the wound site. Conventional commercial bandages and wound dressings fall short in addressing these complexities. To address this issue, we have developed silk-based composite bandages tailored for wound healing. In this study, metal substituted cerium oxide nanoparticles (M-CNPs), and microRNA-146a (mir146), when incorporated into silk composites, can serve as antimicrobial, anti-inflammatory, and antioxidant components in a wound covering product.

Methods: The M-CNPs synthesized by a wet chemical method. The mic146 encapsulated alginate (SA)-chitosan(C)-collage (Col) beads were fabricated using encapsulation equipment. M-CNPs/silk composite films were initially fabricated by a solution casting method and then mic146 beads infused into M-CNPs/silk film to produce a novel composite bandage for wound healing. Fabricated materials were then characterized via FTIR, UV-Vis, X-ray photoelectron spectroscopy (XPS), SEM and TEM; in particular, to confirm nanoparticle and miRNA incorporation. The composite film was cultured with HUVEC cell for 1 and 3 days and then analyzed for cell death/proliferation using WST-1 assay. In addition, a commercial angiogenesis assay kit was used to analyze angiogenesis induction by fabricated films in HUVEC cultures.

Results: XPS determined that Ce4+/Ce3+ ratio and amount silver incorporation are essential in observed antioxidant and antimicrobial properties by incorporated nanoparticles. M-CNPs maintained their ROS scavenging abilities following composite incorporation. In vitro study, release of miRNA and M-CNPs and antioxidant were determined using UV-Vis technique and commercial kit. The cell studies showed no significant cytotoxicity for fabricated bandage treated cells. Further, we observed an increase in angiogenic network formation for fabricated bandage film. Based on this result, we propose that the tested fabricated bandage film can be effective in reducing the ROS, microbial infection, inflammation, and increasing angiogenesis; thus, accelerating the healing rate of wounds.
Conclusion: The results demonstrate that fabricated bandage film can provide combinational therapy deliverable to wound sites. The results showed substantial efficacy in scavenging ROS, limiting microbial infection, inflammation, and enhancing angiogenesis. To the authors’ knowledge, the studies material is the first description of a wound healing bandage to incorporate miRNA technology.

P100 | What We Learned From 683 Cases Of Surgery Plus Hypofractionated Radiotherapy For Keloids: A Single Institutional Experience

Tae Hwan Park
Plastic and Reconstructive Surgery, Hallym University Dongtan Sacred Heart Hospital, Hwaseong-si, Korea (the Republic of)

Keloid treatment is challenging. From May 2021, we have treated 683 keloids with surgery combined with hypofractionated radiotherapy. We have used several strategies in our case series. Complete excision followed by primary closure in single or multiple stages, complete excision plus local flap coverage in a single stage, intralesional excision with biopsy punch device with/without closure, triple combination therapy, debulking surgery, postoperative single dose radiotherapy or two fractions, and closed incisional negative pressure wound therapy is one of those. Through this presentation, we would like to give you valuable insights into the beautiful orchestration of this combined surgery plus hypofractionated radiotherapy approach.

P101 | Association Of Novel Therapies For Complex Chronic Wounds

Debora C. Sanches-Pinto1, Cristina C. Houmsi2, Mario J. Warde3, Rolf Gemperli1
1Plastic Surgery and Burns, HCFMUSP, Sao Paulo, Sao Paulo, Brazil;
2Plastic Surgery, Hospital Edmundo Vasconcelos, Sao Paulo, SP, Brazil;
3Plastic Surgery, Hospital Alemão Oswaldo Cruz, Sao Paulo, SP, Brazil

Background: Chronic wounds are those present for more than 4 weeks failing to produce anatomical and functional skin integrity. They are predominantly a condition of old individuals are with chronic illness. We also face challenges in aesthetic surgery. Methods and Results: 4 chronic complex wound showing the association of new technologies to accelerate the healing process. Case 1: 65 years old (yo) woman, type II diabetes, dialytic with recurrence of a lesion at his right foot already partially amputated. At the first procedure we reduced the ulcer at a minimum size, after full debridement and local flaps we filled all empty spaces with Endoform® and used V.A.C. After one week, full granulation, were able to graft. After 3 weeks total, the flap became fully integrated and the patient was back to his job. Case 3: 65 yo old woman with a donor site infected with a multiresistant germ. After the debridement we used Endoform® with antimicrobial, Silvercel® and Mepilex Border®. The patient also received systemic antibiotics. After 3 weeks the wound was totally closed. Case 4: a paraplegic 66 yo man with an ischiatic pressure sore, treated several times before by other teams, inclusive ours, 3 years ago, successfully. This year he appeared with a lesion similar to an excoriation. We found a huge seroma inside a fibrotic capsule and we made all the investigation to rule out osteomyelitis. After closing the wound with local muscle flaps, there was still slough at the edge of the skin. We covered it with Endoform® and Prevena Plus®. We obtained good results both at the incision and around it. After 3 weeks the patient got back to work. Conclusion: after a correct diagnosis made by multidisciplinary team, we sometimes must gather novel technologies to give our patient as much comfort as possible, discharge faster and be able to make the follow up on an ambulatory basis, with the best cost benefit results, not only considering the money spent but also the quality of life to our patients.

P102 | Acupuncture For Wound Healing: A Review Of Evidence And Its Biological Basis

Charles Shang
University of California, Davis, Nevada City, CA

Purpose: Review the current evidence on the effectiveness of acupuncture for wound healing and its biological basis.

Background: Organizers are small groups of cells which control growth and differentiation of a larger region. Their structure and function have been well established in embryogenesis. Organizers are singular points of morphogen gradient field and bioelectric field. They can be activated by nonspecific subtle stimuli such as needle prick causing long lasting growth control effects. The organizer model of postembryonic growth control suggests that a network of organizers continues to exist after embryogenesis and plays a critical role in regulating tissue growth and repair. Acupuncture points (acupoints) likely originate from the organizers. Acupuncture has been shown to have extensive growth control effects and can promote wound healing. Organizers and acupoints have been confirmed to share many similarities as predicted.

Methods: A literature search was conducted in Pubmed and Google Scholar to identify relevant studies using keywords (acupuncture OR acupoint) AND (wound healing). Studies were included if they met the at least one of the following criteria: (1) they were randomized
controlled trials (RCTs); (2) they investigated the effect of acupuncture on wound healing; (3) they reported outcomes related to wound healing, such as wound size, healing time.

**Results:** Several studies including RCTs on both human and dogs met the inclusion criteria. The results of the studies showed that acupuncture was more effective than sham acupuncture in promoting wound healing in a variety of wound types. The effect sizes were small to moderate, but statistically significant. Acupuncture can promote wound healing by increasing the release of interleukins, the expression of growth factors such as vascular endothelial growth factor and transforming growth factor-β1, and regulating phosphatidylinositol-3-kinase/protein kinase B as well as mitogen-activated protein kinase.

**Conclusions:** Acupuncture can be effective for wound healing by activating the organizers of growth control and increasing the wound healing response. Further research is needed to fully understand the mechanisms by which acupuncture promotes wound healing and to determine the optimal therapeutic parameters for wound healing. The development of wearable medical devices with low intensity transcutaneous acupoint stimulation can improve the cost effectiveness of wound healing.