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Wound Repair and Regeneration — WILEY

ABSTRACT

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2022 Annual Meeting of the Wound Healing Society SAWC-Spring/WHS Joint Meeting

April 6 - 9, 2022

2022 WHS ABSTRACTS

WHS SESSION I: YOUNG INVESTIGATORS SYMPOSIUM

I.01. | Comparing Transcriptomes Of Paired Acute And Chronic Wounds

Harrison J. Shawa[1], Alexander Merleev[1], Alina Marusina[1], Nicholas R. Hum[2], Aimy Sebastian[2], Gabriela G. Loots[2], Sara Dahle[3], Emanuel Maverakis[1], Rivkah Isseroff[1]

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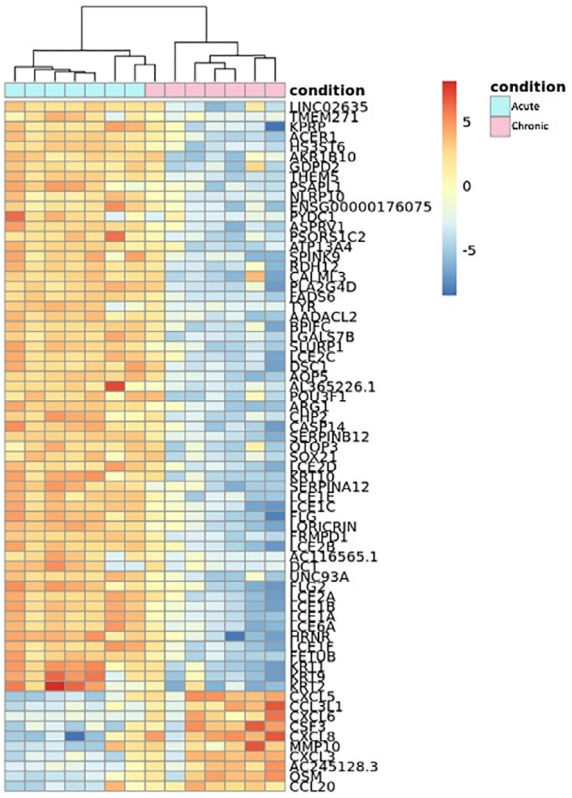
Chronic wounds remain a challenge for the United States healthcare system, yet the molecular pathogenesis of chronic, nonhealing wounds remains to be fully elucidated.

Here, we analyzed the transcriptomic signatures of paired acute and chronic wounds collected from the same patients, same site, and same time to accurately delineate the molecular differences between chronic, nonhealing wounds and acute wounds. Patients included 7 male individuals aged 55 to 69 years with chronic, nonhealing, full-thickness diabetic foot ulcers. Tissue was collected from the chronic wound edge at the time of amputation. 10 minutes thereafter, tissue was collected from the amputation wound edge (acute wound). RNA was extracted and next generation RNA-seq was performed on an Illumina NextSeq 500.

In total, 1,698 differentially expressed genes were identified. Gene set enrichment analysis revealed significant differences cytokine-cytokine interaction (68/294 genes; p<10-13), arachidonic acid metabolism (16/63 genes; p<0.001), linoleic acid metabolism (10/29 genes; p<0.001), IL-17 signaling (20/93 genes; p<0.001), NF-kappa B signaling (20/95 genes; p<0.001), alpha-linolenic acid metabolism (9/25 genes; p<0.001), ether lipid metabolism (11/47 genes; p<0.001), TNF signaling (19/110 genes; p<0.005), and histidine metabolism (7/23 genes; p<0.005). Analysis of the most up- or down-regulated genes indicated differences in keratinocyte proliferation and differentiation, neutrophil and lymphocyte attraction, and extracellular remodeling in chronic wounds. Additionally, differences were detected in dopamine, epinephrine, norepinephrine, and serotonin metabolism and signaling. Analysis of the immune-cell landscape using CIBERSORT algorithm inferred increased numbers of M1 macrophages (9.4-fold; p=0.02), plasma cells (2.2-fold; p=0.02), resting mast cells (86-fold; p=0.003) and activated NK cells (259-fold; p=0.005), while increased numbers of M0 macrophages (2.5-fold; p=0.01), activated dendritic cells (9-fold; p=0.04) and mast cells (7.2-fold; p=0.006), and resting NK cells (9.7-fold; p=0.008) were detected in chronic wounds. Additionally, resting dendritic cells were present in 5 of 7 acute wounds and were absent in each chronic wound.

This comprehensive, same-site, paired acute and chronic wound analysis indicates the presence of dysregulation of immune response, catecholamine signaling, epithelialization, and tissue remodeling in chronic wounds, providing specific targets to heal chronic wounds or prevent acute wounds from becoming chronic.

NRH, AS and GGL were supported by the USDOE, LLNL (DE-AC52-07NA27344).



Heat map of most up- or down- regulated genes (>50-fold change)

I.02. | Healing Phase-Specific Signatures Of Wound Fibroblasts And Matrix Patterns Define Cancer-Associated Fibroblast Subtypes And Predict Cancer Outcome

Mateusz S. Wietecha[1], David Lauenstein[1], Sybille Seiler[1], Juyoung Jin[1], Andreas Goppelt[2], Manfred Claassen[3], Mitchell Levesque[4], Reinhard Dummer[4], Sabine Werner[1]

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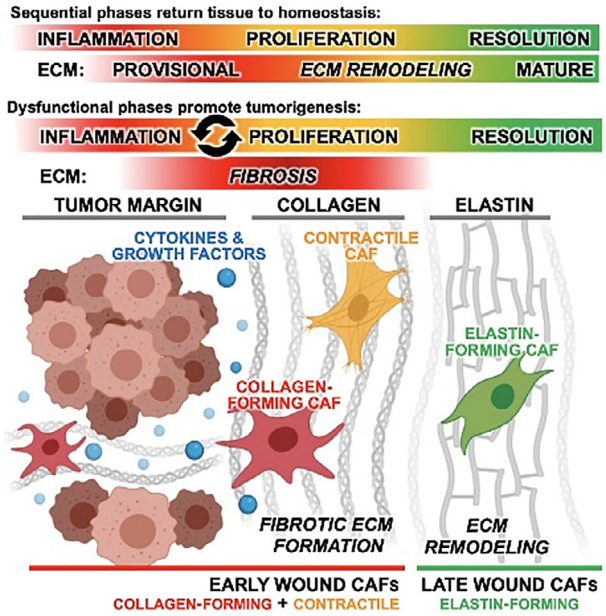
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Background: Cancer encapsulates the wound healing process becoming chronically dysregulated and has been described by Dr. Harold Dvorak as a "wound that fails to heal". Despite the long-standing academic interest in the cellular and molecular parallels between healing and carcinogenesis, a comprehensive molecular comparison between wounds and tumors in vivo is still missing, and the specific roles of the wound healing phases have largely been overlooked. Therefore, we performed a comprehensive multi-omics comparison of wounds and tumors at the whole tissue and single cell levels.

Methods: We first deconstructed the complex wound healing spectrum via a new peak detection bioinformatics package, yielding healing phase-specific gene expression signatures in mice and humans that we then used to enrich healing phases within thousands of samples across twelve cancer types based on The Cancer Genome Atlas (TCGA). We used machine learning to identify prognostic healing phase-predictive signatures followed by pathway enrichment analyses. We performed comprehensive meta-analyses of bulk and single cell transcriptomes of wound- and cancer-associated fibroblasts (CAFs) that we further integrated with published spatial transcriptomics datasets. To determine the translational relevance of our findings, we validated our bioinformatics data by high-throughput extracellular matrix (ECM) imaging of a primary melanoma tissue microarray (TMA).

Results: The most phase-predictive and prognostic genes in the TCGA enriched in ECM-related pathways and pointed toward fibroblasts as key drivers of these signatures. Meta-analyses of wound fibroblasts and CAFs revealed healing phase-related CAF subtypes across several cancer types that expressed divergent ECM repertoires within spatially-defined niches. An "early-wound" CAF subtype localized to the inner tumor stroma, correlated with poor prognosis, and enriched for collagen-related ECM genes. In contrast, a "late-wound" CAF subtype localized to the outer tumor stroma, correlated with better prognosis, and enriched for elastin-related ECM genes. ECM imaging and analysis of the primary melanoma TMA linked healing-associated CAF subtypes and their divergent collagen- vs elastin-rich matrix signatures and architectures with patient survival and cancer recurrence.

Conclusion: The results identify healing phase-specific genes and matrix patterns with prognostic potential in cancer, and pave the way for the development of innovative wound healing- and matrix-based diagnostic tools to predict and potentially influence cancer outcome.



I.03. | Adipocytes Transition To Pro-Fibrotic Fibroblasts And Contribute To Muscle Fibrosis Following Nerve Injury

Amanda F. Spielman, Michelle Griffin, Nicholas Guardino, Heather E. desJardins-Park, Kristian E. Bauer-Rowe, Jason L. Guo, Jennifer Parker, Darren B. Abbas, Derrick Wan, Michael T. Longaker
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Background: Recent studies have suggested that interconversion between adipocytes and fibroblasts (the cellular mediators of skin scarring) occurs in the wound environment. Muscle fibrosis occurs due to aging, muscle injury, and muscular dystrophies causing weakness and pain. The role of adipocytes in muscle fibrosis has not been fully explored. Our study examines whether mature adipocytes participate in muscle fibrosis following nerve injury via conversion into dermal fibroblasts.

Methods: AdipoqCre transgenic driver mice were crossed to R26mTmG reporter mice to generate AdipoqCre;ROSA26mTmG mice, in which mature adipocytes (Adipoq/adiponectin-expressing cells) express green fluorescent protein (GFP), and all other cells express a red fluorescent protein (RFP). AdipoqCre;ROSA26mTmG mice underwent sciatic nerve injury per established protocol. Injured tibialis anterior and gluteus maximus muscle were harvested at postoperative day-14 (POD-14) and underwent immunofluorescent staining for fibroblast (collagen type IV/col-IV, alpha-smooth muscle actin/a-SMA) and adipocyte (perilipin) cell markers. To achieve local adipocyte ablation, AdipoqCre;ROSA26mTmG;R26tm1(HBEGF)Awai mice were generated and wounded, and diphtheria toxin (DT;400ng/mouse) was injected into the muscle at the nerve injury site. DT- and vehicle control-treated muscle underwent histologic analysis.

Results: We identified a significantly greater number of adiponectin-lineage-positive cells (GFP+) within injured muscle at POD-14 compared to uninjured muscle (*P<0.05,n=5). The GFP+ cells that infiltrated the muscle injury exhibited upregulation of fibrotic markers including col-IV and α-SMA, and downregulation of adipocyte markers including perilipin, indicating conversion of adipocytes into pro-fibrotic fibroblasts. DT-induced ablation of Adipoq-lineage-positive cells resulted in reduced muscle scarring, with decreased collagen deposition compared to vehicle-control injured muscle (*P<0.05,n=5).

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Conclusion: Our study demonstrates that adipocytes undergo conversion to pro-fibrotic fibroblasts in response to nerve injury and contribute to muscle fibrosis. Targeted adipocyte ablation resulted in reduced muscle scarring, suggesting that adipocyte-derived fibroblasts are important contributors to muscle fibrosis.

I.04. | Deferoxamine Improves The Wound Microenvironment And Improves Muscle Regeneration In A Mouse Model Of Pressure Ulcers

Nurul Jannah M. Nasir[1], Johannes Heemskerk[1], Peter T. So[2], Lisa Tucker-Kellogg[1]

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Introduction: Deferoxamine (DFO) is an FDA-approved iron-chelating drug that has been found to accelerate skin wound closure, but its effect on muscle and its mode of action are unclear. This study aims to investigate the therapeutic effect of DFO on the wound microenvironment and subsequent muscle regeneration following pressure ulcers in a transgenic mouse model.

Methods: Multicolour (confetti) fluorescent protein expression was induced in muscle satellite stem cells and their progeny by crossing inducible Pax7cre and Brainbow2 transgenic C57BL6 mice. This construct causes newly regenerated muscle to express confetti fluorescent proteins while the pre-existing tissue remains dark. Pressure ulcers were created in the mice by applying a pair of 12 mm magnets to the dorsal skinfold and panniculus carnosus muscle, in two intervals of 12 hours. Mice with PU were subcutaneously injected with DFO in the treatment group and 0.9% saline in the control group (n=7 each). Ex vivo wound tissues were analysed at different time-points: 3, 10, 40 and 90 days after injury.

Results: DFO treatment caused an eight-fold decrease in free iron ($p < 0.01$) compared to saline control, according to Perl's Prussian blue staining. At 3 days following injury, DFO treatment caused a two-fold decrease in lipid peroxidation ($p < 0.001$) and DNA oxidation ($p < 0.05$), and a five-fold decrease in extracellular traps ($p < 0.001$). At 10 days post-pressure injury, DFO-treated wound tissues had two-fold higher angiogenesis ($p < 0.05$) and two-fold greater infiltration of alternately activated macrophages ($p < 0.01$), than saline-treated tissues. At 40 days and even 90 days following PU, saline-treated muscle tissues failed to regenerate or were partially regenerated, with deformed and split fibers, while DFO-treated tissues exhibited larger volumes of regenerated muscle ($p < 0.01$), and fewer malformations in myofiber morphology ($p < 0.0001$).

Conclusions: In summary, we show that DFO causes a decrease in oxidative stress, and promotes granulation and infiltration of pro-regenerative macrophages. Furthermore, DFO improved the extent and quality of muscle regeneration following pressure ulcers. Given that pressure ulcers are a huge healthcare burden with an unmet need for clinical interventions, our study advances DFO towards clinical trials for PU treatment.

I.05. | Diabetic Dysfunctional Exosomes (Diaexosomes) Impair Resolution Of Human Wound Inflammation

Poornachander Guda[1], Adam Anthony[2], Anu Sharma[1], Andrew Course[2], Sashwati Roy[1], David Clemmer[2], Chandan K. Sen[1], Subhadip Ghatak[1]

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Background: Paracrine effects via exosome emerged as the primary mechanisms of cell-cell crosstalk at the wound-site. Resolution of wound inflammation relies on successful crosstalk between keratinocytes that must re-epithelialize, and the blood borne wound-site macrophage, which must mount and timely resolve inflammation. We tested the hypothesis that wound fluid diaexosomes impair resolution of wound inflammation.

Methods: Of the different cell types that are present in wound-edge, keratinocytes originate from ectoderm and are uniquely characterized by presence of intermediate filament keratins. Two independent databases (Vesiclepedia and Exocarta) identified type I keratin 14 (Krt14) as unique to exosomes originating from keratinocytes. Using magnetic Krt14 immunoprecipitation method, we successfully separated exosomes of keratinocyte origin from a heterogenous pool of extracellular vesicles (EV) in human wound fluid of 17 non-diabetic healing and 32 diabetic patients. Diaexosomes were characterized and reported in EV-track. The multiplexing direct stochastic optical reconstruction microscopy (dSTORM) technology (resolution 20nm) were used to quantitatively analyze the abundance of miRNA cargo in hExoκ and surface modification at single exosome resolution.

Results: Human keratinocyte-derived exosomes (hExoκ) were isolated from wound fluid and characterized as per EV-track guidelines. The size of hExoκ ranged from 75-105 nm. Super resolution dSTORM microscopy demonstrated abundance of Krt14 partner Krt5 in hExoκ. The hExoκ compromises of 10% of total exosome pool and 1% of the total EV present in wound fluid normalized with albumin. Compared to non-diabetic subjects, there was a significant reduction of hExoκ in wound fluid of diabetic subjects. Co-immunofluorescence staining of heterogeneous ribonucleoprotein (hnRNP) A2B1 and Q, which are critical for miRNA packaging in hExoκ showed significant low abundance in wound-edge keratinocytes of diabetic subjects. dSTORM microscopy using molecular beacons with Cy5 reporter and BHQ3 quencher demonstrated abundance of pre-miR-21 in hExoκ that were positively correlated with wound closure. Unlike hExoκ diaexosomes of diabetic subjects, addition of hExoκ isolated from non-diabetic subjects to macrophages resulted in conversion to a pro-resolution phenotype.

Conclusion: This work provides a novel insight into the pathophysiological mechanism of diaexosomes of chronic wound patients. Hyperglycemia dysregulates exosome packaging, release, and surface modification of keratinocyte originating diaexosomes. These perturbations compromise crosstalk between keratinocyte and wound macrophages complicating resolution of wound inflammation.

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I.06. | Characterization of Mechanoresponsive Inflammatory Cells during Wound Healing

Kellen Chen, Michelle Griffin, Dominic Henn, Clark A Bonham, Katharina Fischer, Jagannath Padmanabhan, Artem A. Trotsyuk, Dharshan Sivaraj, Melissa C. Leeolou, Hudson C. Kussie, Savana L. Huskins, Sydney Steele, David Perrault, Michael T. Longaker, Geoffrey C. Gurtner

Stanford University, Stanford, CA, United States

Background: Repair after tissue injury involves a dynamic interplay among not just tissue resident cells (e.g., fibroblasts), but also cells recruited from the circulation. Myeloid cells, such as monocytes and macrophages, are derived from hematopoietic precursors and migrate to sites of injury where they play a role in modulating all stages of wound healing and scar formation. There is mounting evidence that mechanical stimuli are also able to modulate monocyte and macrophage response during tissue healing, but the exact mechanisms behind this "mechano-immunomodulation" remain incompletely understood.

Methods: We attached a mechanical strain device to the mouse dorsum to initiate a uniform and consistent strain profile across an incisional wound to create hypertrophic scar (HTS) formation in mice. To investigate mechano-responsive immune cells, we performed parabiosis of wildtype (WT) and GFP+ mice, allowed the mice to develop a shared blood circulation, initiated HTS formation in the WT mouse, and analyzed the cells using single cell RNA sequencing (scRNA-seq), fluorescent-activated cell sorting (FACS), and immunofluorescent staining.

Results: Mechanical modulation significantly upregulated the presence of inflammatory subtypes within the healing tissue, characterized by an increase in infiltrating GFP+ cells from 5.4% to 12.2%. In the GFP+ circulating cells, mechanical strain directly increased the proportion of fibrotic myeloid cells, primarily defined by the monocyte marker Ly6c2 as well as the TGFB responsive and macrophage activating gene Thbs1. Mechanical strain also increased the proportion of inflammatory myeloid cell populations, defined by Ccl and Il6 chemoattractants, and Cd74+ migratory myeloid cells. Utilizing both a pharmacological blocker of focal adhesion kinase (FAK) as well as a myeloid specific FAK knockout (KO), we demonstrated that modulating mechanical signaling abrogated those responses and instead promoted homeostatic myeloid transcriptional fates.

Conclusions: Tissue injury activates a cascade of signaling pathways to recruit and orchestrate various cell types during healing. Our study indicates that modulating mechanical stress directly affects myeloid cell phenotypes and interactions with other cell types in the complicated, multicellular milieu of wound healing. This principle has been previously unexplored in the context of fibrosis and regeneration, with most previous studies focused on fibroblast heterogeneity and transcriptional profiles. To our knowledge, this is the first study to directly investigate the effects of modulating mechanotransduction on immune cell response at the single cell level utilizing parabiosis and wound healing. Collectively, we demonstrate that mechano-immunomodulation of the "early responders" of healing can trigger a cascade of downstream regenerative healing.

I.07. | Overcoming Radiation Induced Oral Fibrosis Through The Down Regulation Of WNT Signaling Using BMP-7 Inhibitors

Nicholas Guardino, Michelle Griffin, Amanda F. Spielman, Darren B. Abbas, Megan King, Jennifer Parker, Derrick Wan, Michael T. Longaker

Surgery, Stanford, Menlo Park, CA, United States

Background: Oral scarring is a common debilitating side effect of head and neck radiation therapy. With no effective treatment patients suffer long-term pain, taste dysfunction, and infection from radiation induced oral fibrosis. Previous work has suggested that Wnt signaling may contribute to radiation scaring in the oral mucosa. We have developed a preclinical in vivo murine model to study the effects of radiation damage on the oral mucosa. Our study evaluates the effects of Wnt pathway inhibitors to reduce scarring in the oral mucosa following irradiation.

Methods: Adult C57Bl/6 mice (6–8-week-old) (n = 6) were randomized to one of three conditions: 1. Treated with 30 Gy external beam irradiation to the oral mucosa delivered as six fractionated doses of 5Gy over a period of 12 days (irradiation group), 2. Irradiation to the oral mucosa with simultaneous treatment with a Bone Morphogenetic Protein 7 (BMP-7) inhibitor (BMP-7) (treatment group), and 3. No irradiation and no treatment (control group). Mice were euthanized at 4 weeks post irradiation for histological and flow cytometry analysis. Oral sections underwent immunofluorescent staining for fibrosis (e.g., collagen type I/col-I, alpha smooth muscle actin/a-SMA), vascularization (CD31 staining), inflammation (F4/80 antigen), and Wnt signaling (e.g., axin2, Wnt1) markers and were imaged on a confocal microscope.

Results: Histological analysis by H&E staining confirmed dermal thickening and increased collagen content in the irradiated group compared to the control group ($p < 0.05$), confirming the reproducibility of the murine model to induce oral fibrosis. BMP-7 inhibitor treatment decreased the dermal and epidermal thickening observed in the irradiated group ($p < 0.05$). The treatment group also demonstrated reduced dermal fibrosis with decreased col-I and a-SMA staining compared to the irradiation group ($p < 0.05$). Flow cytometry analysis further showed reduced numbers of profibrotic fibroblasts (CD26+) in the treatment compared to the irradiation group ($p < 0.05$). Immunofluorescence staining confirmed significantly high levels of Wnt pathway protein signaling (Axin2, Wnt1) in the irradiation group compared to the treatment and control group ($p < 0.05$). Mice receiving the BMP-7 inhibitor also demonstrated improved vascularization as shown by CD31 staining and a decreased inflammatory infiltrate evidenced by F4/80 macrophage staining ($p < 0.05$).

Conclusions: Our novel murine model suggests that canonical Wnt signaling may be responsible for oral scarring post irradiation. Targeting Wnt signaling through BMP-7 inhibitors may be an effective method to reduce the burden of radiation induced oral fibrosis.

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I.08. | A Double-Blind Randomised Placebo-Controlled Clinical Trial Demonstrates That Pre-Emptive Priming Of Human Skin With Topical Epigallocatechin-^{3}-Gallate Improves Cutaneous Skin Scarring

Sara Ud-Din[1], Traci A. Wilgus[2], Douglas McGeorge[3], Ardeshir Bayat[1]
[1]*The University of Manchester, Manchester, United Kingdom.* [2]*The University of Ohio, Columbus, OH, United States.* [3]*Grosvenor Nuffield Hospital, Chester, United Kingdom*

Epigallocatechin-3-gallate (EGCG) enhances cutaneous wound healing due to its antiangiogenic, anti-inflammatory, and antioxidant properties. We previously demonstrated the role of EGCG in scarring in ex vivo human scar models and in a double-blind randomised placebo-controlled clinical trial which showed that immediate and delayed application of this anti-scarring topical improved skin scarring by reducing scar thickness and mast cell count and increasing hydration and elasticity. The concept of pre-emptive priming of skin pre-injury offers a novel approach in optimizing cutaneous scarring outcome. The objective was to deliver an active compound at the optimal time in order to maximize its impact and improve cutaneous scarring. Therefore, pre-emptive application of anti-scarring topical, EGCG, pre-injurycompared with post-injury can potentially be superior on scarring outcome. Thus, we conducted another double-blind randomised placebo-controlled trial comparing the effects of pre-emptive priming of skin with EGCG topical pre-injuryversus post-injury. Healthy volunteers (n = 40) were split into 4-groups; each undergoing different modes of application versus placebo: Group-1 = priming (7Days) pre-injury, Group-2 = priming (3D) pre-injury, Group-3 = immediate (0D) day-of-injury, Group-4 = delayed application (14D) post-injury. Excisional skin-biopsies in upper-arms were evaluated weekly with multiple non-invasive objective quantitative devices over 8-weeks. Histological, immunohistochemical, mRNA sequencing and QRT-PCR studies were performed on tissue-biopsies. EGCG reduced mast cells at weeks-4 and 8 by gene and protein analyses ($p < 0.01$). Group 1 was superior to other groups ($p < 0.01$) in both clinical (blood flow) and laboratory parameters (elastin and immune marker expression). Additionally, there was down-regulation of angiogenic-markers by mRNA-sequencing and of CD31 and VEGF-A at weeks-4 and 8 ($p < 0.01$) by immunohistochemistry and at week-4 ($p < 0.05$) by QRT-PCR. EGCG increased antioxidant levels (HO-1) at week-4 ($p < 0.01$). Immunohistochemical analysis of elastin was up-regulated at week-8 in EGCG-treated samples compared to placebo samples in all groups ($p < 0.01$) The greatest differences between groups were in group-1 ($p < 0.01$). This was also supported by clinical elastin measurements in group-1 at week-8 ($p = 0.029$). In conclusion, pre-emptive priming of skin pre-injury has significant beneficial effects on skin scarring shown by reducing mast cells, blood flow and angiogenesis plus increasing elastin content.

I.09 | Macrophage Acute Contact Induces Fibroblast Intracellular Stress In A Calcium- And Gap-Junction-Dependent Process

Maya Ezzo[1,2], Jun Bo Wang[1], Gilbert Pecoraro[1], Joao Firmino[1], Pardis Pakshir[1], Boris Hinz[1,2]
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Rationale: Fibrosis (organ scarring) is responsible for >40% disease-related deaths worldwide. Key effector and target cells in fibrosis are fibroblasts activated to myofibroblasts to produce and contract collagen into detrimental scar tissue. We published fibroblasts make contact with mechanically attracted macrophages (Mϕ) and found that first contact with Mϕ results in fibroblast contractile events. However, the mechanisms of contact-induced acute fibroblast contraction are unknown.
Hypothesis: Touching Mϕ initiate fibroblast contraction and acute intracellular stress by inducing an increase in intracellular calcium levels.
Objective: Quantify fibroblast contractions and calcium transients before, during, and after contact with Mϕ, with and without connexin-43 (Cx43) formation.
Methods: The change in fibroblast stress was quantified before, during, and after contact formation with Mϕ by measuring nuclear translocation of mechanosensitive transcription factor YAP. In addition, changes in fibroblast contractions were computed from the displacement of embedded fluorescent beads on a collagen matrix. Fluorescent reporters were used to detect contact-induced cytosolic calcium changes as fast indicator of contractile response. As one possible source of calcium, we inhibited Cx43 gap junctions formed between Mϕ and fibroblasts through siRNA knockdown of Cx43. Gap junctions allow intercellular passage of small molecules such as tracer molecule calcein-AM. Both, calcium dyes and gap-junction permeable tracers were assessed by immunofluorescence and flow cytometry.
Results: Fibroblasts in short contact with Mϕ exhibited 1.5-fold higher nuclear levels of YAP and 1.7-fold increased contraction speed compared to control fibroblasts. Increased fibroblast stress upon Mϕ contact correlated with a change in frequency and 1.6-fold enhanced intensity of cytosolic calcium transients. Fibroblasts and Mϕ both expressed Cx43 and exchanged calcein-AM indicating formation of hetero-cellular gap junctions that in principle – allow passage of calcium. Consequently, knockdown of Cx43 in co-cultures of fibroblasts and Mϕ affected the regularity and frequency of fibroblasts calcium increases.
Conclusion: Our results show Mϕ contact formation enhances fibroblast cytoskeletal stress and contractile activities in a calcium- and Cx43 channel-dependent process. We propose the molecular components that mediate Mϕ-fibroblast interaction as potential targets for anti-fibrosis strategies

WHS SESSION J: CONCURRENT ORAL ABSTRACTS I

J1: Biomaterials in Wound Healing And Tissue Regeneration

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J1.01 | Encapsulation of MSCs in Self-Assembling Peptide Hydrogels with Activity Against Methicillin-Resistant Staphylococcus aureus (MRSA) and Pseudomonas aeruginosa (Pa) Promotes Faster Wound Closure and Improves Tissue Regeneration in a Clean and Pathogen-Contaminated Type I Diabetic (STZ-induced) Mouse Model

Eliza B. Hoobler[1], Yewoo Lee[1], Isabelle Eldridge[1], Naomi Young[2], Evangeline Cornwell[2], Manav Mehta[1], Ana Tellechea[1], Nava Rijal[1]
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About one-fifth of diabetes-related hospitalizations are the result of complications from delayed wound healing and pathogen colonization, leading to ~100,000 non-trauma-induced lower-limb amputations per year and ~35,000 deaths in the US each year due to the rise of antibiotic-resistant strains of bacteria. The objective of this research is to develop a flowable cell delivery scaffold to promote tissue regeneration in clean and pathogen-contaminated diabetic wounds.

Hypothesis: This shear-thinning-self-assembling-peptide gel matrix (G4matrix) encapsulated with mesenchymal stem cells (MSCs) enhances implanted cell survival and promotes healing in both clean and pathogen-contaminated diabetic wounds vs. control (PBS + MSCs). **Methods:** 5-mm full-thickness, splinted, excisional wounds (n= 50 mouse, 2 wounds/mouse) are created on the dorsum of each mouse. Tissues were collected at days 2 and 21 post-wounding for further analysis.

Results: The G4matrix used in this research are effective against Pa and MRSA. We demonstrated complete clearance of 106 CFU (MRSA) and complete clearance of 106 CFU (Pa) which was significantly ($p<0.0001$) higher than a non-antimicrobial matrix control. To confirm cell viability, we encapsulated MSCs in G4 matrices for 24 hours and used a luciferase-based assay to quantify cell viability using Relative Luminescence Units (RLU). We demonstrated significantly higher cell viability in G4matrix ($p<0.0001$) compared to gelatin, our positive control, and PBS. MSCs encapsulated in G4matrix resulted in significant ($p < 0.02$) reduction in the wound area of non-contaminated wounds at day 21 versus control (PBS + MSCs). Similarly, at day 21, wounds were found to have complete healing with immature epithelium over the center of the wounds for clean wounds. Furthermore, H&E staining of sectioned wound tissue from pathogen-contaminated wounds demonstrated that the wounds treated with G4matrix + MSCs have robust granulation tissue and re-epithelialization with significantly lower edema ($p <0.001$) at days 2 and 21, and significantly lower leukocyte infiltrate levels ($p < 0.02$) at days 2 vs. controls.

Conclusion: G4matrix + MSCs improved wound closure in non-contaminated and decreased markers of inflammation in contaminated wounds as compared to untreated controls.

J1.02 | 3D Hydrogel System For Tailoring Stem Cell Biologics To Improve The Re-Epithelialization Of Wounds

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Statement of Purpose: Inadequate re-epithelialization by Keratinocytes (KCs) results in incomplete wound closure and progression towards chronic, non-healing wounds. Chronic wounds have an improper balance and composition of bioactive compounds within the tissue, resulting in a cycle of sustained, inappropriate cellular signaling and inadequate neotissue formation. Therefore, there remains a critical need for a therapy that restores the appropriate re-epithelialization capacity of wounds and reestablishes proper wound healing signaling to prevent progression of chronic wounds. Recent investigations have demonstrated that the diverse milieu of secreted factors from Adipose-derived Mesenchymal Stem Cells (ASCs) could offer a unique approach to treating chronic wounds. **Objective:** Investigate the ability to tailor ASC secretome in 3D culture with fibrin-priming.

Methodology: Human ASCs were expanded in either traditional 2D culture or a novel 3D system. The 3D system is a customizable bioprinted hydrogel that is mechanically similar to soft tissue (i.e. adipose). The role of fibrin in modulating ASC secretive activity to promote re-epithelialization was evaluated. Throughout expansion, Conditioned Medium (CM) was collected, saved, and analyzed via a Proteome microarray, HPLC-MS/MS, and ELISAs. ASCs were assessed for senescence and phenotypic changes. KCs were treated with ASC-CM and assessed for changes in re-epithelialization at an RNA, proteomic, and functional level.

Results: Expansion of ASCs in 3D resulted in ~70% reduction in senescence and ~1.5x increased expression of ASC "stem-like" markers, relative to 2D. Proteomic microarray of 55 secreted proteins in ASC-CM indicated that 3D expansion significantly increased the secretion of ~15% of the proteins, including the EGF, HGF, IGF, and FGF protein families. Additionally, a KC scratch assay demonstrated ~1.5x increase in migration and proliferation of KCs when treated with ASC-CM from 3D culture, relative to 2D. Moreover, fibrin-priming of ASCs resulted in ASC-CM that further enhanced KC proliferation and migration, relative to without fibrin-priming in culture. Additional proteomic experiments evaluating ASC-CM after fibrin-priming are ongoing.

Conclusion: Together, this data demonstrates the potential of a novel 3D hydrogel system to enhance the regenerative capacity of ASCs and permit the priming of ASCs to tailor secretory components. Thus, these studies provide insight towards advancing the development of tailored biologics for future regenerative therapeutics.

J1.03 | A Novel Fibromodulin-Releasing Granular Hydrogel For Functional Tendon Injury Recovery

Zhong Zheng[1], Xue Xu[2], Yulong Zhang[1], Pin Ha[1], Chenshuang Li[3], Emily Yen[4], Benjamin Wu[1], Andrew Da Lio[1], Kang Ting[5], Chia Soo[6]

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Background: The tendon is one of the most commonly injured tissues in the body and when ruptured, can lead to permanent dysfunction and a diversity of complications. Although many efforts have been devoted to seeking a safe and efficient treatment for enhancing tendon healing, the treatments available rarely gain a satisfactory prognosis because of poor tissue quality, inferior mechanical properties because of fibrotic scar tissue, and chronically reduced functionality. Here, an injectable granular hydrogel that releases fibromodulin (FMOD) - a bioactive extracellular matrix (ECM) that enhances cell mobility – was developed in response to the need for new strategies in initiating endogenous tissue repair, accelerating wound healing, and direct functional restoration of the damaged tendons.

Materials and Methods: Firstly, the biopotency of FMOD on rat tenocytes was investigated in vitro regarding cell proliferation, migration, invasion, and ECM expression. Next, the storage and loss modulus, the swelling ability, and the viscosity of the engineered injectable FMOD-releasing granular hyaluronic acid (FMOD/gHA)-hydrogel were characterized. Moreover, the efficacy of the FMOD/gHA-hydrogel was determined in a transected rat's Achille's tendon injury model. Power analyses by GPower predicted the sample size to ensure that = 0.05 and power = 0.8. Parametric data were compared by one-way ANOVA and two-sample t-tests while the Mann-Whitney U test and Kruskal-Wallis ANOVA tests were used for non-parametric data.

Results and Discussion: The granular hydrogel has excellent water-absorption properties capable of maintaining the administrated FMOD with enough viscosity to stick to tissue surfaces and to fill the arbitrarily-shaped tendon defects. Moreover, the FMOD/gHA-hydrogel displays a burst-to-sustained two-phase release of FMOD. Thus, a single administration of the FMOD/gHA-hydrogel is sufficient and is then followed by a constant dose-maintaining period. Furthermore, the FMOD/gHA-hydrogel significantly augmented tendon healing histologically, mechanically, and functionally. Particularly, the breaking strength of the wounded tendon and the gait performance of treated rats returns to the same normal level as the healthy controls. FMOD is a native ECM component that is broadly distributed in the connective tissue and multiple HA hydrogels have been approved for human usage. Therefore, FMOD/gHA-hydrogel has a promising future as a therapeutic agent in the clinical setting.

Conclusion: A new and effective FMOD/gHA-hydrogel is developed in response to the urgent demand for tendon healing strategies.

J1.04 | Application of No Releasing Gel Increases Fibronectin, TGF-β1, and Accelerates Wound Healing in Diabetic Mice

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Surgery, Stanford University, Stanford, CA, United States

Background: According to the American Diabetes Association (ADA), over 9-12 million patients suffer from chronic ulceration each year which costs the healthcare system over 25 billion annually. There is a significant unmet need for new and efficacious therapies to accelerate closure of nonhealing wounds. Nitric Oxide (NO) plays an important role as a messenger molecule during wound repair. NO levels typically increase rapidly after skin injury in the inflammatory phase and gradually diminish as wound healing progresses. The molecular mechanisms of how increased NO concentration affects wound healing and leads to re-epithelialization and wound closure remains incompletely understood. In this study, we sought to investigate the effect of local administration of an NO-releasing gel on excisional wound healing in diabetic mice.

Methods: We utilized 15-week-old C57BL/6 mice with leptin receptor deficiency in a splinted excisional wounding model to mimic human wound healing through deposition of new granulation tissue and re-epithelialization rather than contracture. Excisional wounds of each mouse received either NO-gel or control phosphate buffered saline (PBS)-gel treatment twice daily until full wound closure (N=5 mice per group). The wound areas were quantified and expressed as a percentage of the original wound area. To assess angiogenic signaling and vasculature in the healed wounds, sections were stained for fibronectin and TGF-β1.

Results: Topical administration of NO releasing gel significantly accelerated the rate of wound healing as compared with PBS treated mice. The mean time for complete wound healing was 14.0 ± 0.75 days in the NO gel treated group compared to 16.0 ± 0.75 days in the PBS-treated group (*p < 0.05). At day two and day seven of healing, as well as after wound closure, we found that immunofluorescent staining for fibronectin and TGF-β1 was significantly increased in the NO treatment group compared to the control group.

Conclusions: We have shown that application of NO releasing gel rapidly upregulates fibronectin and TGF-β1 to accelerate closure, facilitate improved ECM reconstruction, and promote tissue regeneration in chronic impaired wounds. The results of this work may have important clinical implications for the management of patients with non-healing wounds.

J1.05 | A Tissue-Engineered Cellular Construct Polarizes Macrophages Towards A Complex M1-M2 Phenotype

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StrataGraft is a cellular and/or tissue product (CTP) consisting of a stratified keratinocyte-containing epidermis and fibroblast-embedded collagen dermis that promotes healing in patients with deep partial-thickness burns. The mechanisms behind these effects are poorly understood. Macrophages, the primary cell type of the innate immune system, regulate tissue repair by shifting their phenotype from a pro-inflammatory population (M1) to a diverse population, collectively referred to as M2, that orchestrate healing. Because of their success in promoting healing, we hypothesized that CTPs promote this phenotype transition of macrophages. The purpose of this study was to analyze the crosstalk between CTPs and macrophages and to investigate the mechanisms behind the effects.

To determine how the components of the CTP influence macrophage phenotype, primary human macrophages (N=4 donors) were co-cultured directly with a CTP, indirectly with a CTP via separation by transwell inserts, directly on the collagen scaffolds used in CTP fabrication, or on low-activation plastic as an unactivated control. After 1 and 3 days, macrophages were stained with an 8-marker flow cytometry panel to characterize phenotype: M1-related markers (CCR7, CD80, CD38 and PD-L1) and M2-related markers (CD206, CD209, CD163 and CXCR4), or RNA was isolated to measure the expression of 72 genes related to inflammation and wound healing by NanoString. Multidimensional analysis was used to identify unique macrophage populations using FlowSOM. Statistical analysis of individual marker expression or cell composition within clusters was performed with one-way ANOVA.

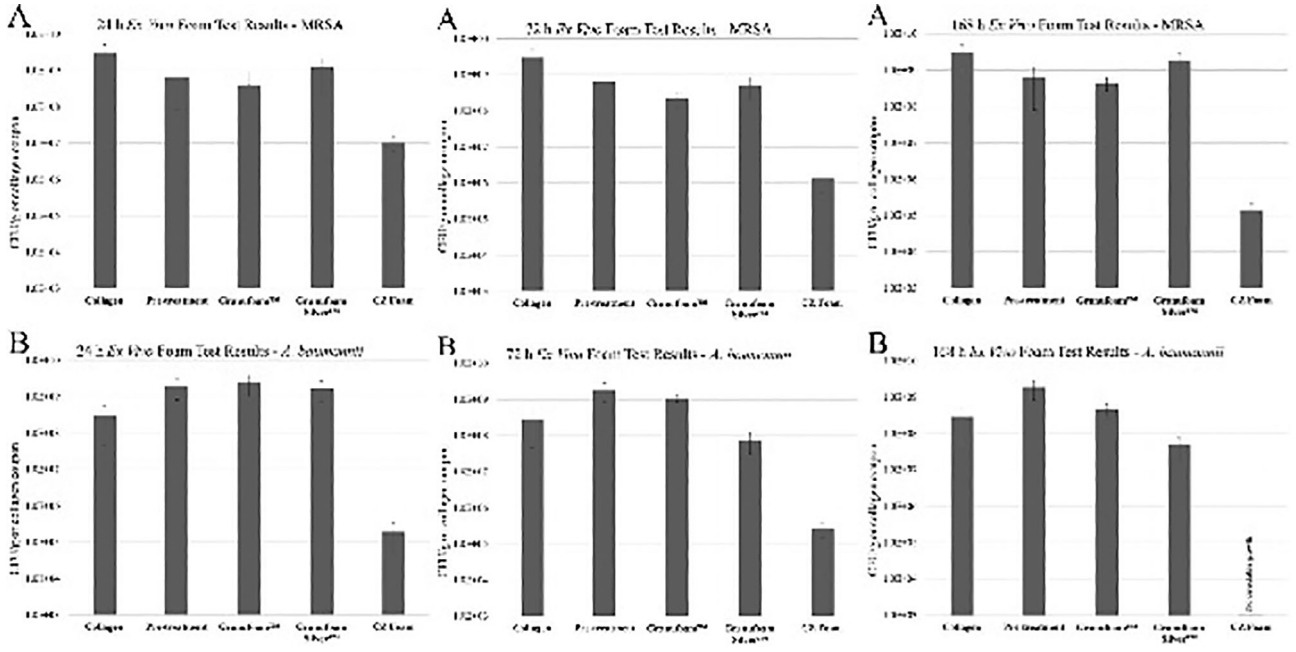
Flow cytometry showed that macrophages reduced M1 marker expression when in direct contact with CTPs or acellular collagen for 1 day. By day 3, M2 markers were upregulated only when in direct contact with CTPs. Multidmensional flow cytometry and gene expression analysis uncovered more complex phenotypes in macrophages cultured in direct contact with CTPs, including upregulation of some M1 genes, including IL6 and TNFA, in addition to increased M2 marker expression. These results suggest that the components within CTPs influence macrophage polarization, in that the collagen scaffolding decreases M1 marker expression, while the cellular component of CTPs is necessary for M2-like polarization. However, macrophages also take on unique hybrid phenotypes. Further studies are required to determine the influence of these effects on wound healing.

J1.06 | Ex Vivo And In Vivo Analysis Of A First-In-Class Antibioifilm Polyurethane Foam For Use In Negative Pressure Wound Therapy

Isaac J. Bell[1], Kaden Rawson[1], Travis Neuberger[1], Tyler Smith[1], Spencer Brown[1], Paul Sebahar[2], Ryan Looper[2], Paul Pasquina[3], Brad Isaacson[1], John Shero[4], Dustin Williams[1]

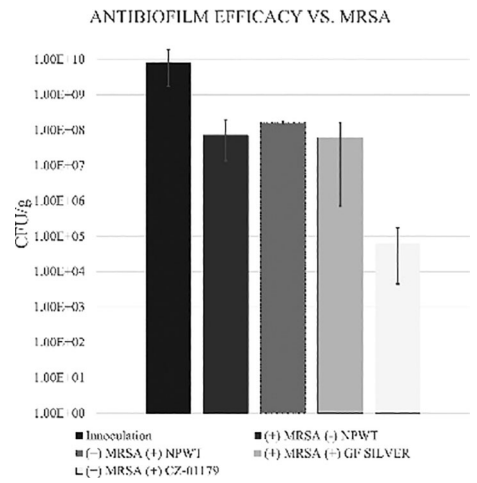
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Background: Negative pressure wound therapy (NPWT) is a prevalent treatment for traumatic, battlefield-related injuries. Wound contamination often occurs since greater than 75% of military wounds test positive for a pathogenic isolate at the time of injury. Furthermore,

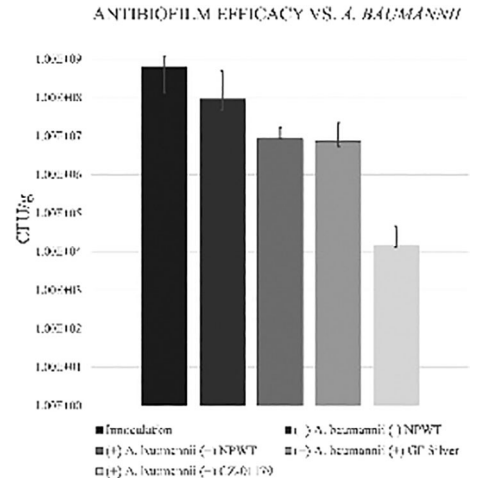


Top row: MRSA treated with NPWT in ex vivo porcine excision wound model at 1, 3, and 7 day intervals. Bottom row: A. baumannii treated with NPWT in ex vivo porcine excision wound model at 1, 3, and 7 day intervals.

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Left to right: Inoculation, Positive Control, PU vs MRSA, GRANUFOAM Silver vs MRSA, 10% w/w CZ-01179 vs MRSA



Left to right: Inoculation, Positive Control, PU vs A. baumannii, GRANUFOAM Silver vs A. baumannii, 10% w/w CZ-01179 vs A. baumannii

the open-cell structure of polyurethane (PU) foam used in NPWT can potentially harbor and encourage increased biofilm growth at the wound site. To date, GRANUFOAM Silver by KCI is the most commonly used variation of commercially-available antimicrobial foam for NPWT. However, silver nanoparticles are minimally effective against biofilms. The purpose of this project is to incorporate a biofilm-specific bactericide into a PU foam matrix that is capable of reducing biofilm bioburden in an ex vivo and in vivo porcine excision wound model.

Methods: We developed a PU foam with a 10% w/w concentration of CZ-01179 (anti-biofilm agent) within a hydrogel scaffold to match and compare against GRANUFOAM Silver. (Minimum inhibitory concentration values and antibiofilm efficacy of CZ-01179 were previously validated using an in vitro broth assay.) Ex vivo testing was performed by inoculating full-thickness pig skin explants with MRSA and A. baumannii biofilms, followed by a two-day incubation period. The skins were then treated with GRANUFOAM Silver or 10% w/w CZ-01179 in the presence of a 125-mmHg wound vac for 1, 3, and 7 day increments. In vivo testing was conducted on Yorkshire pigs. 4x4 cm excision wounds were made on the epaxial muscles and inoculated with MRSA and A. baumannii biofilms. NPWT treatment with GRANUFOAM Silver or 10% w/w CZ-01179 began 2 days post-surgery and continued for 7 days. Upon completion of the ex vivo and in vivo trials, the wounds were harvested, and the bioburden within the granulation tissue was assessed with a 10-fold dilution to determine the colony forming units per gram of tissue.

Results: Ex vivo testing with 10% w/w CZ-01179 resulted in a 2.5 log10 (1 day), 3.2 log10 (3 days), and 4.1 log10 (7 days) reduction against MRSA and 3.5 log10 (1 day), 3.5 log10 (3 days), and 8.5 log10 (7 days) reduction against A. baumannii when compared to the control. GRANUFOAM Silver expressed a < 1 log10 (1 day), 1 log10 (3 days), and < 1 log10 (7 days) reduction against MRSA and < 1 log10 (1 day), 1.5 log10 (3 days), and 1.5 log10 (7 days) reduction against A. baumannii when compared to the control. For each ex vivo trial, n = 6 data points were obtained. After the 7 day treatment, in vivo testing for GRANUFOAM Silver vs A. baumannii revealed 1.1 log10 reduction and < 1 log10 reduction against MRSA when compared to their respective controls. However, treatment with CZ-01179 for the same period of time expressed a 3.7 log10 reduction against A. baumannii and a 3.1 log10 reduction against MRSA. For each in vivo trial, n = 6 wounds.

Conclusions: Ex vivo and in vivo testing indicated greater antibiofilm efficacy against MRSA and A. baumannii in comparison to GRANUFOAM Silver.

J2: Cells at the Center of Wound Healing

J2.01 | Senescent Adipose-Derived Stem Cells Stimulate Inflammatory Phenotype Macrophages via Monocyte Chemo Attractant Protein-1

Anesh Prasai[1], Jayson W. Jay[1], Ye Wang[1], Andrew Murton[1], Steven E. Wolf[2], Amina El Ayadi[1]

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Background: The use of autologous adipose-derived stem cells (ASCs) in cosmetics has become widespread in elderly adults. ASCs impart therapeutic properties via balancing the macrophage polarization; however, limited studies have investigated the interaction between aged ASCs and macrophages. ASCs from aged subjects constitutively secrete higher levels of inflammatory factors such as the monocyte chemotactic protein1 (MCP1), which may promote macrophage polarization towards an inflammatory phenotype. We hypothesize that aged ASCs alter macrophage polarization.

Method: ASCs were isolated from 24 months old, and four months young Wistar rats, with five animals in each cohort. ASCs were characterized and quantified for staining with beta-galactosidase (beta-gal), a

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marker of senescence. MCP1 was measured in the conditioned media of both groups using an ELISA assay. Both old and young ASCs were challenged for 3 hours with 100 ng/ml lipopolysaccharide (LPS). Fluorescently-labeled (PHK67) human monocytes (THP1 cells) were treated with 12myristate 13-acetate (PMA) to promote macrophage differentiation. After differentiation, the interaction between THP1 cells and old and young ASCs ± MCP-1 was examined 24 hours after co-culture.

Result: Both aged and young ASCs displayed cell surface markers associated with stemness. However, aged ASCs had significantly higher β-gal staining and constitutively secreted higher MCP1 levels compared to young ASCs ($p<0.05$). Senescent ASCs expressed significantly higher levels of inflammatory transcripts (NF-kB, IL-1b, GM--CSF, CCL5, IL6, and TLR4) 3 hours after treatment with LPS compared to the young ASCs. THP1 cells showed increased migration towards aged ASCs and in the presence of MCP-1 (100ng/mL) and had a significantly higher inflammatory phenotype expression compared to young ASCs ($p<0.05$).

Conclusion: Co-culture pre-incubated with MCP1 antibody showed reduced expression of THP-1 staining in both young and old ASCs. MCP-1 increases the migration and inflammatory phenotype expression in THP-1 co-cultured with aged ASCs; an effect that was negated by the MCP1 antibody. The immunomodulatory properties of senescent ASCs may impair the beneficial effects of ASCs when derived from older subjects.

J2.02 | The Effects Of Inhalational E-Cigarette Exposure On Acute Wound Healing In A Surgical Flap Murine Model

Amy Anne D. Lassig[1], Anna Wilson[1], Connor Dedeker[1], Bruce Lindgren[2], Marissa Macchietto[2], Davis Seelig[2], Dorothy Hatsukami[2]
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[2]*University of MN, Minneapolis, MN, United States*

Introduction: While the prevalence of cigarette smoking is falling, the rate of electronic cigarette use is rising exponentially, especially among youth. These products are assumed to be less harmful than combusted cigarettes but have been shown to result in vascular and pulmonary inflammatory dysregulation. There is little evidence, however, on the effects of e-cigarettes on acute wound healing, and thus we sought to investigate these effects in a pilot investigation using a murine surgical flap model.

Methods: C57Bl6 mice were exposed to inhalational exposure of Juul e-cigarette, University of Kentucky research combusted cigarette, or control, 5 days per week for 4 consecutive weeks. A standard dorsal McFarlane flap was elevated and repaired, and acute wound healing was evaluated over the subsequent 21 days. Wound healing was prospectively assessed by the following outcomes: flap survival / tissue necrosis via digital images / Image J, perfusion via thermography / laser doppler, tissue structure via standard histology immunohistochemistry, and gene expression via RNA sequencing.

Results: Combusted cigarette and electronic cigarette exposed mice had evidence of diminished inflammatory cell infiltrates in the inflammatory stage of wound healing (day 3) with diminished perfusion and increased flap necrosis at day 7. Inflammation persisted in combusted and electronic cigarette exposed animals beyond the anticipated inflammatory stage to lead to diminished perfusion and flap survival.

Conclusions: Electronic cigarettes result in dysregulated inflammation, diminished perfusion, and diminished flap survival in acute surgical wounds that is less severe than combusted cigarettes but distinct from controls. In light of the widespread use of these products, especially in young populations that frequently experience traumatic wounds, additional investigation is needed on the effects of electronic cigarettes on acute wound healing.

J2.03 | Alterations Of The Wound-Induced Senescence Response In Aging

Maria Shvedova, Rex Jeya Rajkumar Samdavid Thanapaul, Grace H. Shin, Jack Crouch, Daniel S. Roh
Division of Plastic and Reconstructive Surgery, Department of Surgery, Boston University, Boston, MA, United States

The aim of this study was to compare wound-induced senescence in young and old mice to determine if there are differences influencing wound healing.

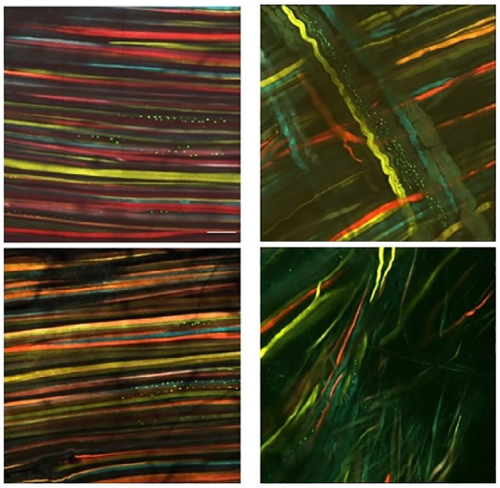
Young (2 months old, n=20) and old (23-24 months old, n=30) C57BL/6 (wild type) male mice underwent a single 1cm surgical dorsal full-thickness skin wound. Mice were monitored every 3 days with digital imaging. Mice were euthanized at 6, 12, 18, and 24 days, and the healing wound tissue (including the surrounding 1cm2 skin area) was excised and analyzed by qPCR for senescence markers (p16 and p21) and senescence-associated secretory phenotype (SASP) markers, staining for senescence-associated beta-galactosidase (SA-β-gal), and p21 immunostaining. Statistical analysis was performed using t-test for comparison between young and old and ANOVA and post-hoc tests. $p<0.05$ was considered statistically significant.

Wound contraction in old group was significantly delayed compared to young at each timepoint. qPCR analysis revealed significant increase in p16 expression in young group at day 6, and rapid return to the baseline by day 12. Old mice did not mount any significant increases in p16 expression. Young mice demonstrated increased expression of p21 peaking at day 18, while no p21 increase was registered in old group. In young mice, the expression of SASP factors IL-6, MCP-1, MMP3, MMP-9, and TGF-β, significantly increased at day 6 and returned to the baseline by day 12. In old mice, only IL-6 was increased, and no significant increases in MCP-1, MMP-9 or TGF-β were registered, while MMP-3 increase occurred later at day 12. Young but not old mice demonstrated significant increase in SA-β-gal-positive cells starting from day 6, followed by decrease by day 24. p21 immunostaining revealed 2-3x fold increase in p21-positive cells starting from day 12, peaking at day 18 in young but not in old group.

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Newly regenerated muscle fibers, expressing fluorescent proteins.

Non-fluorescent capillaries appear as thin, dark streaks over the muscle. The confetti construct encodes four fluorescent proteins, chosen at random during Cre-Lox recombination: yellow cytoplasmic, red cytoplasmic, cyan membrane-localized, and green nuclear-localized fluorescent proteins. Scale bar: 200 microns.



Myofibers regenerated after cardiotoxin injury

Myofibers regenerated after a pressure ulcer

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In conclusion, young mice demonstrate a robust but transient wound-induced senescence response with shorter healing time. In contrast, the senescence response to wounding in old mice is significantly altered which may contribute to delayed wound healing observed in aging. Further studies are warranted to determine the identities of wound-induced senescent cells in young and old wound healing; and determine the mechanisms which drive decreased wound-induced senescence in aging.

J2.04 | Failed Phagocytosis During The Impaired Healing Of Pressure Injuries

Nurul Jannah M. Nasir, Johannes Heemskerk, Lisa Tucker-Kellogg
Duke-NUS Medical School, Singapore, Please select an option below, Singapore

Background: We aimed to compare the regenerative sequence in acute wounds versus chronic wounds. For this comparison, we created an animal model of delayed wound-healing using pressure injuries of muscle. This model does not rely on diabetes or infection to impair the normally-fast regeneration of mice.

Methods: In a specific-pathogen-free facility, mice received an acute wound (cardiotoxin injection into panniculus carnosus muscle) or a chronic wound (magnet pressure injury of the panniculus carnosus muscle and dorsal skinfold). Newly regenerated tissue was distinguished from pre-existing regions via inducible heritable fluorescence.

Results: Acute injuries showed normal phases of wound healing -- at 3 days, dead tissue had been cleared; at 10 days, immature muscle fibers filled the wound bed; at 16 days, mature muscle fibers filled the injured region with near-native morphology.

In contrast, pressure injuries showed a failure of phagocytosis to clear necrosis after 3 days, and histology showed immune infiltration around, but not inside, the compressed region. Immune cell remnants were detected in the compressed region by immunostaining for extracellular traps. Extracellular traps are toxic anti-microbial structures produced by expulsion of nuclear DNA from dying neutrophils or macrophages.

At 10 days after pressure-induced injury, dead tissue was extruded to slough, and viable immune cells filled the wound bed. At 40 days, immature myofibers were present, but only at the wound margins, and they had poor morphology, including wavy, branched, and non-parallel fibers. At 90 days, unregenerated gaps still remained in the muscle layer.

Conclusion: Pressure injuries displayed inadequate phagocytosis and wholesale slough of all layers. Some phagocytes did infiltrate the region of dead tissue, but despite the absence of pathogens or infection, they died enacting aggressive anti-microbial cytotoxicity. Compared with toxin-induced injuries, pressure injuries had worse speed, extent, and quality of muscle regeneration. Similar mechanisms of impairment may occur in human pressure injuries, which also exhibit slough and poor regeneration.

J2.05 | The Oral Mucosa Hosts Distinct Fibroblast Subpopulations to Facilitate Regenerative Wound Repair

Michelle Griffin[1], Jessica Cook[2], Dario Bofelli[2], Nicholas Guardino[1], Amanda F. Spielman[1], Michael Januszyk[1], Kellen Chen[1], Darren B. Abbas[1], Rachel Zwick[2], Ophir Klein[2], Michael T. Longaker[1]
[1]Stanford university, Palo alto , CA, United States [2]University of California, San Francisco, CA, United States

Background: The oral mucosa is a unique tissue that displays regenerative scarless healing following wounding in under 3 days. Growing evidence has aimed to characterize the fibroblasts responsible for scarring following skin injury in the hope to modulate skin scarring and fibrosis. However, the identification and behavior of oral mucosal fibroblasts (OMFs) that facilitate regeneration are unknown. This

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observation presented a unique opportunity to study the molecular events differentiating oral regeneration from skin scarring.

Methods: To identify the OMF subpopulations responsive to dermal wounding, we examined the transcriptional responses of OMFs to mechanical injury in a mouse model. Adult C57Bl/6 mice received matched 2-mm wounds within their buccal mucosa and facial skin. At 1-, 2-, 4- or 7-days post-wounding, mice were euthanized and whole tissues were prepared for single-cell RNA sequencing, including unwounded samples. Using R and Python software packages, we defined the fibroblast subtypes in oral wounds in comparison to skin wounds at all timepoints. Furthermore, we identified and defined the cross-talk between cell types in oral and skin wounds using network analysis platforms.

Results: Our dataset identified dynamic changes in the cell composition of the wound environment in the oral and skin tissue including the epithelial, mesenchymal and immune populations following wounding. Our analysis revealed that the fibroblast cells clustered differently in the oral and skin tissue following injury. Cluster analysis reveals five subpopulations of fibroblasts with unique cluster markers in the oral tissue following wounding. Pseudotime analysis revealed that the fibroblasts following skin and oral injury followed distinctly unique transcriptional trajectories. Cell-cell interactions were also notably different between the oral and skin tissue with specific upregulation and downregulation of pathways as a result of wounding in the oral tissue.

Conclusions: These data suggest that oral tissue displays regeneration following wounding due to its unique resident population of fibroblasts. Studies to examine and mimic the behavior of these oral regenerative fibroblast subpopulations hold promise to unlocking the mechanism of scarless wound repair.

J2.06 | Elevation Of Baseline Skin Senescence Delays Wound Healing

Rex Jeya Rajkumar Samdavid Thanapaul, Maria Shvedova, Grace H. Shin, Jack Crouch, Daniel S. Roh

Division of Plastic and Reconstructive Surgery, Department of Surgery, Boston University School of Medicine, Boston, MA, United States

The purpose of this study was to determine how states of elevated skin senescence, such as what occurs in aging and chronic wounds, influences subsequent wound healing. CAG-luciferase transgenic C57BL/6 mice were used to isolate and culture primary ear fibroblast cells. Senescence was induced using gamma irradiation (10Gy), and cellular senescence was confirmed using senescence-associated beta-galactosidase (SA-β-gal) staining. Subcutaneous cell transfers (1x106 cell suspensions) with irradiated or non-irradiated fibroblast cells were performed on young C57BL/6 male mice (2 months) (n=20). The viability of transplanted cells after cell transfer was confirmed using in vivo Bioluminescence Imaging on days 0 and 7. Skin tissue harvested 7 days after cell transplantation was used to examine gene expression of senescence markers (p16, p21) and SASP factors (IL-6, MCP-1, MMP-3, MMP-9, and TGF- β), SA- β -gal activity, and p21 immunohistochemistry. Cell transfers with irradiated and non-irradiated fibroblasts were performed on additional mice (n=12), followed by wounding with a 1cm surgical full-thickness excisional wound on the dorsal skin. Digital imaging was used to monitor wound healing daily, and wound contraction was calculated using ImageJ. Senescent changes, such as altered cellular morphology, were observed, and more than 70% of irradiated cells were SA-β-gal positive, compared to 2% of non-irradiated cells. After 7 days of cell transfer, the skin of mice with irradiated cell transfer had statistically significant increases in the expression of senescent markers and multiple SASP factors, SA- β -gal positive cells, and p21 positive cells compared to mice receiving non-irradiated cell transfer. Young mice treated with irradiated cell transfers had significantly delayed wound healing compared to non-irradiated mice that were persistent after wounds were healed. This delayed wound healing was similar to that seen in naturally aged 2-year-old mice. Using our subcutaneous cell transfer model of increasing skin senescence in young mice we believe that elevated baseline skin and tissue senescence may cause delays in wound healing in aging and also in chronic wounds. Efforts to modify chronic senescence may be targets to improve wound healing in these groups.

J3: Fibrosis and Scarring

J3.01 | Pixel Grafts Can Be Expanded Up To ^{5}00 Times To Re-Epithelialize A Burn Wound

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Introduction. Skin graft expansion in major burns is restricted to a 1:6 expansion ratio (possibly 1:9) by current methods. For this reason, skin transplants are limited by donor site availability for patients with extensive burns, who may have only small areas of healthy skin for use as donor sites. The purpose of this project is to demonstrate the efficacy of pixel grafting, to produce split-thickness skin grafts (STSG) and dermal grafts which can be expanded at least 100 times (1 cm2 donor skin will cover 100 cm2).

Methods. Deep partial-thickness burns were created on the dorsum of Yorkshire pigs. Three days post injury the burns were debrided and grafted with epidermal/dermal and dermal pixel grafts using expansion ratios from 1:2 to 1:500. The pixel grafts were prepared by harvesting 0.3 mm thick skin grafts from the same donor site going down in depth (Layer 1: STSG; Layer 2: dermal graft). Subsequently, the grafts were minced to 0.3 x 0.3 x 0.3 mm pixel grafts, suspended in a small volume of hydrogel and transplanted onto debrided burn wounds. Healing was monitored up to 28 days and biopsies were collected on days 6, 10 and 14. The wounds were excised for histologic analysis. In addition, to measure wound area reduction and wound depth, wound images were taken.

Results. Histology demonstrated that when both STSG pixel grafts and dermal grafts were transplanted onto porcine full-thickness

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wounds in a 1:2 ratio they were able to fully re-epithelialize the wounds in 14 days. At day 14, re-epithelialization-% using other expansion ratios varied from 46 % (1:500) to 64 % (1:10) and by day 28 all the wounds were fully re-epithelialized. Wound images obtained at different time points showed that by day 28 all the pixel graft grafted wounds had reduced in area efficiently, from 70 % (dermal pixel grafts 1:50) to 81% (STSG 1:100). No statistically significant differences were observed between the pixel graft study groups and STSG (standard of care).

Conclusions. This study concluded that both epidermal/dermal and dermal pixel grafts can be suspended in a hydrogel and transplanted into wounds regardless of orientation (dermal side up or down), making the transplantation greatly simplified.

J3.02 | Microfibril Associated Protein 5 Regulates Skin Fibroblast Function and Scar Formation

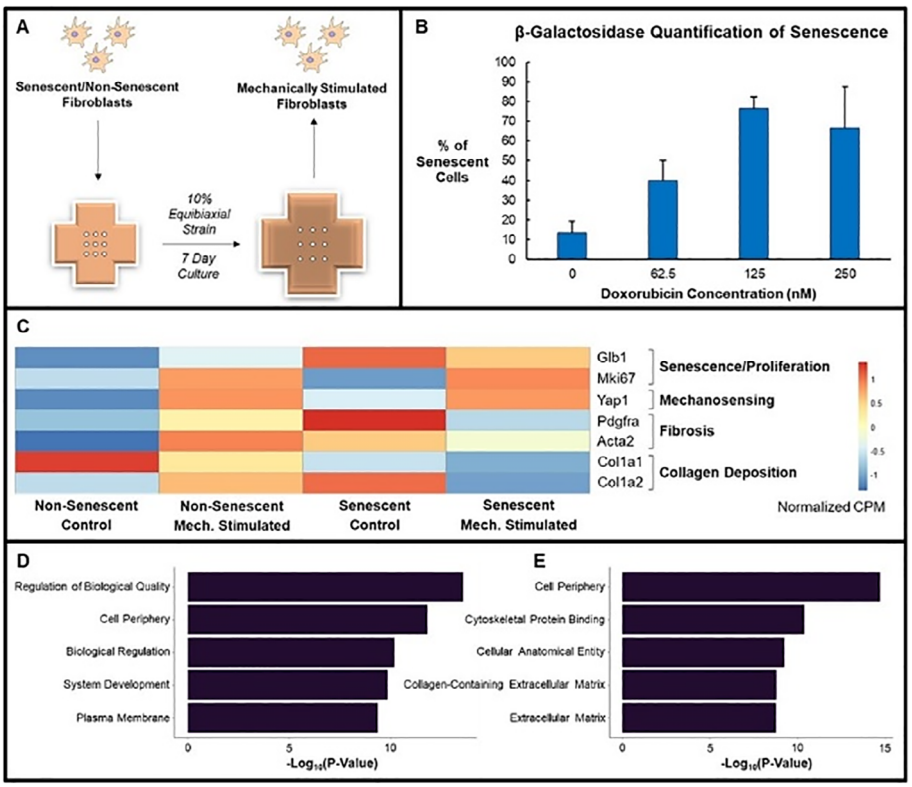
Chen Han[1], Lin Chen[1], Mohamad Altakriti[1], Junhe Shi[2], Sammy Ferri-Borgogno[3], Samuel C Mok[3], Luisa A. DiPietro[1]

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Recent studies in our lab have demonstrated that Microfibril Associated Protein 5 (MFAP5, or microfibril-associated glycoprotein2/MAGP2) is upregulated in healing skin wounds. MFAP5 is a 25 kD extracellular matrix (ECM) glycoprotein that is increased in many fibrotic diseases including idiopathic pulmonary fibrosis, systemic sclerosis, lung, and skin fibrosis. However, the role of MFAP5 in wound healing and scar formation is unknown. The aim of this study was to investigate the effects of MFAP5 on fibroblast function in vitro and to examine the effects of antibody blockade of MFAP5 on mouse skin wound healing. In vitro, the addition of recombinant MFAP5 to dermal fibroblasts caused significantly enhanced fibroblast migration and upregulation of mRNA expression of α-SMA, collagen I, collagen VI, MMP1, MMP9 and TGFß-1 when assessed by real time-PCR. Treatment of murine 5mm excisional skin wounds with anti-MFAP5 monoclonal antibody (clone 130A) had no effect on wound closure. However, compared to control, Antibody treated wounds exhibited significantly decreased collagen deposition at day 21 as analyzed by Masson's Trichrome staining. Additionally, immunohistochemical staining demonstrated that MFAP5 antibody treated wounds showed significantly decreased angiogenesis (measured by CD31+ staining) at day 7. Taken together, the results suggest that MFAP5 exerts a profibrotic effect in the healing wound by enhancing fibroblast migration, increasing the number of myofibroblasts, and modifying ECM synthesis.

J3.03 | Mechanical Stimulation Reverses Pro-Fibrotic Transcriptional States in Senescent Fibroblasts

Jason L. Guo, Michelle Griffin, Nicholas Guardino, Kellen Chen, Geoffrey C. Gurtner, Michael T. Longaker



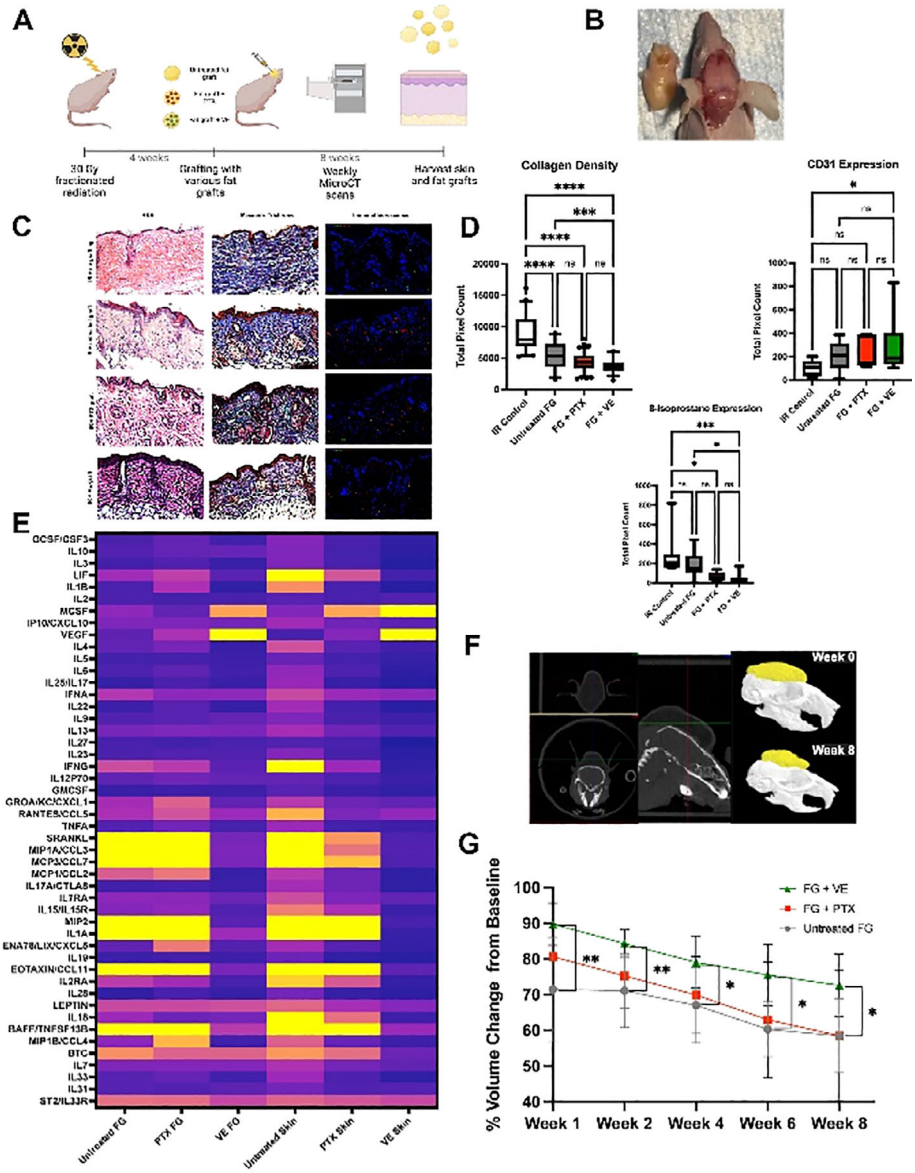
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Background: Senescent fibroblasts accumulate with age, contributing to fibrosis and impaired healing. It is of longstanding clinical interest to reverse this pro-fibrotic transcriptional state. Interestingly, senescence downregulates mechanotransduction in fibroblasts, but the effects of this phenomenon on skin fibrosis are unclear. We utilized a stretchable collagen I hydrogel (Fig. 1A) to investigate if exogenous mechanical stimulation could reverse the pro-fibrotic transcriptome of senescent fibroblasts.

Methods: Dermal fibroblasts were harvested from C57BL/6 mice by enzymatic digestion and flow cytometry, cultured in growth media, and exposed to 0-250 nM doxorubicin (72h) to induce senescence, quantified by β-galactosidase. Fibroblasts were encapsulated (1,000,000 cells/mL) in 1.92 mg/mL collagen I, 0.8x MEM, and 16mM HEPES. Gels were formed in 20x20x5mm cruciforms and cultured 7 days (media replaced each 2-3 days) with 10% equibiaxial strain for mechanical stimulation. RNA was retrieved by collagenase digestion and TRIzol extraction. RNAseq was performed (40M paired-end reads), aligned to mm10 (HISAT2), and quantified (StringTie). Differential expression analysis and gene ontology (GO) analysis of the top 500 differential genes were performed (edgeR; α=0.05). All experiments performed at n=3.

Results: β-galactosidase showed optimal 76.7% senescence induction with 125nM doxorubicin (Fig. 1B). RNAseq showed increased Glb1 (senescence) and decreased Mki67 (non-senescence/proliferation) expression for senescent fibroblasts, which were reversed by mechanical stimulation (Fig. 1C). Yap1 (mechanosensing) expression increased with mechanical stimulation of non-senescent and senescent fibroblasts. Pdgfra and Acta2 (pro-fibrotic, canonical) were highly enriched in senescent fibroblasts. Critically, these pro-fibrotic markers were reversed by mechanical stimulation in senescent fibroblasts only. Col1a1 was downregulated by senescence and mechanical stimulation, while Col1a2 was downregulated by mechanical stimulation of senescent fibroblasts only.



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GO analysis revealed that mechanical stimulation differentially modified transcriptomes of non-senescent and senescent fibroblasts. The top 5 enriched terms for senescent fibroblasts were characterized by systemic modifications to biological quality/development (Fig. 1D), while the top 5 enriched terms for non-senescent fibroblasts were largely superficial responses to stretched matrix/gel (Fig. 1E).

Conclusions: Mechanical stimulation differentially modified normal vs. senescent fibroblasts, increasing pro-fibrotic transcription for non-senescent fibroblasts but reversing pro-fibrotic transcription and systemically modifying biological qualities for senescent fibroblasts. Furthermore, mechanical stimulation mitigates inherent features of the senescent phenotype (Glb1+/Mki67-). Ultimately, our model suggests that exogenous mechanical stimulation reverses the pro-fibrotic phenotype of senescent dermal fibroblasts.

J3.04 | Vitamin E Treated Fat Grafts Demonstrate Improved Volume Retention And Decreased Radiation-Induced Fibrosis

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Introduction: Fat grafts and vitamin E administration have both previously been described to mitigate radiation-induced skin fibrosis. Fat graft retention continues to hinder its long-term functionality affected mainly by two factors: an initial inflammatory response and chronic necrosis secondary to hypovascularity. Thus, we aimed to simultaneously improve fat graft retention along with radiation-induced fibrosis via local augmentation of fat grafts with cheap, non-toxic, and highly available treatments.

Methods: Ten adult CD-1 nude male mice at 6-8 weeks of age underwent scalp irradiation with 30 Gy fractionated into 6 doses of 5 Gy over a 12 day period. Mice recovered for four weeks after completion of radiation wounding to allow chronic fibrosis to develop (A). After recovery, mice received 1000mL of donor human fat graft to the scalp. Mice were separated into 4 conditions: ungrafted irradiated, untreated fat graft, fat graft treated with pentoxifylline (PTX), and fat graft treated with vitamin E (VE). Fat graft volume retention was monitored in-vivo using microCT scans with 3-dimensional reconstruction and analysis at weeks 0, 1, 2, 4, 6, and 8 after grafting. Fat graft and overlying skin were harvested and underwent histological and cytokine analysis (B).

Results: Histological analysis demonstrated significant improvement in radiation-induced fibrosis, CD31+ expression, and 8-isoprostane expression in the vitamin E group compared to other treatment conditions (C,D). Furthermore, cytokine analysis revealed decreased cell differentiation markers and inflammatory markers within the vitamin E cohort (E). Lastly, from 1 week post-grafting until the end of the study, fat graft volume retention was significantly higher with vitamin E treated grafts compared to the untreated or pentoxyfylline treated grafts (F,G).

Conclusion: Radiation-induced fibrosis and fat graft volume retention are both simultaneously improved with local administration of the fat-soluble vitamin E, improving both cosmesis and functionality of fat grafting in chronically irradiated fields.

J3.05 | Using A Systems Approach To Harness Biological Variance To Identify Pro-Fibrotic Fate Determining Factors

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Background: The current study of wound healing groups subjects independent of where they are in the wound healing process at the time of presentation, and independent of what phase(s) they switch to during the study period. A wound which begins healing, then stalls for 6 weeks, only to go on and eventually heal is compared to wounds which never stall, or which continue to get worse. The wound itself is the central unit for comparison. A new basis for grouping for the purpose of identifying the active processes in wound healing, impairment, or scarring segregates the wounds under investigation on the basis of what they are doing at the time of inquiry. Rabbit cornea heal similarly to humans and have a similar incidence of fibrotic healing. While the incidences are similar, any given cohort does not present with an homogenous scarring response. This internal biological variance in outcomes can be harnessed to improve the study of the molecular systems controlling the divergent tissue fates.

Methods: A rabbit corneal lamellar keratectomy scarring model was used. An imaging technique was employed to discern corneas in the act of scarring versus those which were not. The 6 mm wound and surrounding 2 mm anulus were homogenized and subjected to poly-A mRNA sequencing. The corneas (n = 16) were grouped based on scar formation rates in silicoto select the corneas with the highest (n =3) and lowest (n = 3) rates prior to a differential expression analysis in order to determine factors that differed in those with active scar growth versus those without.

Results: Out of the ~33,000 known transcripts, ~15,000 were found to be expressed. Of those 15,000, 399 transcripts were differentially expressed in corneas with active scar growth. Two unexpected growth factors from the VEGF family (VEGF-A and PLGF) were the most significantly up-regulated growth factors in the actively scaring group (2-fold and 4-fold, respectively $p < 0.0002$). Five immune function-related factors were found to be uniquely present in the active corneas compared to the non-active. Also, general markers for neutrophils such as S100A8/A9 were the highest differentially expressed factors between the two groups (avg 64-fold, p = 1.5 x10-4).

Conclusions: Instead of comparing injured corneas to uninjured corneas, which ends up grouping non-scarring with scarring tissues, and comparing the subgroups of scarring vs non-scarring after post-injury staging, a substantial amount of differences could be highlighted. Two non-canonical factors from the VEGF family were identified despite

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the absence of angiogenesis. This technique can also be used in staging chronic wounds for differential analysis in studying the basic molecular biological differences of wounds exhibiting different behaviors at different times.

J3.06 | Comparative Transcriptome Analysis Of Superficial And Deep Partial-Thickness Burn Wounds In Yorkshire Vs Red Duroc Pigs

Jesse Q. Nguyen[1], Fatemeh Sanjar[1], Sai Lakshmi Rajasekhar Karna[1], Andrea B. Fourcaudot[1], Li-Ju Wang[2], David T. Silliman[1], Zhao Lai[2], Yidong Chen[2], Kai P. Leung[1]

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Hypertrophic scars are a common negative outcome of deep partial-thickness burn wounds resulting in increased dermal thickness, wound area contracture, and inflammation of the affected area. The red Duroc and Yorkshire porcine breeds are common large animal models for studying dermal wounds due to their structural similarities to human skin; however, the porcine transcriptomic profiles of dermal burn wounds and healing process are not well known. In response, a longitudinal transcriptomic comparative study was conducted comparing red Duroc and Yorkshire superficial and DPT burn wounds to their respective control uninjured tissue. Using next-generation RNA-sequencing, total RNAs were isolated from burn wound tissue harvested at 0, 3, 7, 15, 30, and 60 days post-burn and mRNA-seq and gene expression read counts were generated. Significant differentially expressed genes relative to uninjured tissue were defined and active biological processes were determined using gene set enrichment analyses. Additionally, collagen deposition, a-SMA protein concentration, epidermal and dermal thickness measurements, and wound area changes in response to burn injury were characterized. Overall, the red Duroc pigs, in response to both burn wound types, elicited a more robust and prolonged inflammatory immune response, fibroblast migration and proliferation as well as heightened levels of extracellular matrix modulation relative to respective burn types in the Yorkshire pigs. Collectively, the red Duroc deep partial-thickness burn wounds produce a greater degree of hypertrohic scar like response compared to Yorkshire DPT burn wounds. These findings will facilitate future porcine burn studies down-selecting treatment targets and determining effects of novel therapeutic strategies. Some of the data have been submitted to a peer-reviewed journal for consideration of publication.

J4: Genes and Genetic Therapies

J4.01 | A Novel Sshrna-Antimir Combination Therapy For Accelerating Healing Of Diabetic Foot Ulcers

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In diabetic foot ulcers, the normal response to hypoxia is impaired and many cellular processes involved in wound healing are hindered. Central to the hypoxia response is HIF-1a, which activates multiple factors that enhance wound healing. PHD2 regulates HIF-1a activity by targeting it for degradation under normoxia. HIF-1a also upregulates microRNA (miR)-210, which in turn regulates factors involved in cell cycle control, DNA repair, and mitochondrial respiration in ways that are antagonistic to wound repair. We have identified a highly potent short synthetic hairpin RNA (sshRNA) that inhibits PHD2 and an antisense oligonucleotide (antimiR) that inhibits miR-210. Both oligonucleotides were chemically modified to increase biostability and decrease immunostimulatory effects. Using the sshRNA to silence PHD2 transcripts stabilizes HIF-1a and, in combination with the antimiR targeting miR-210, increases proliferation and migration of keratinocytes in vitro. To assess activity and delivery in vivo, PHD2-targeting sshRNAs and miR-210 antimiRs (separately and combined) were formulated for topical delivery to wounds using layer-by-layer (LbL) application to Tegaderm® mesh. The resulting multilayered coating gradually degrades under physiological conditions, releasing sshRNA and antimiR for sustained cellular uptake. Because there is no good animal model of human impaired healing, we employed two different models: db/db mice and an ischemic/neuropathic diabetic rabbit model. In the mouse model, formulated treatments were applied directly to splinted full-thickness excisional wounds. Cellular uptake was confirmed using fluorescent sshRNA. Wounds treated with a single application of PHD2 sshRNA or antimiR-210 closed 4 days faster than untreated wounds, and wounds treated with both oligonucleotides closed on average 4.75 days faster ($p<0.001$). Markers for neovascularization and cell proliferation (CD31 and Ki67, respectively) were elevated in the wound area following treatment, and vascular endothelial growth factor (VEGF) was increased in sshRNA-treated wounds. For evaluation in the rabbit model, New Zealand white rabbits were treated with Alloxan to became diabetic and then treated with LbL-formulated therapeutic RNAs as in the mouse model. A significant increase ($p<0.05$) in extent of wound closure at 8 days post-wounding was seen with antimiR-210 in the diabetic rabbits. Our results suggest that silencing of PHD2 and/or miR-210 by localized delivery of sshRNAs and antimiRs is a promising approach for the treatment of chronic wounds, with the potential for rapid clinical translation.

J4.02 | SERPINB3A Mediates Wound Healing and Tissue Regeneration

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Purpose: We aimed to elucidate a novel mechanism of regulation for partial epidermal EMT in wound healing mediated by Serpinb3a.
Methodology: NCBI Gene Expression Omnibus (GEO) was queried and GEO2R was used to evaluate gene expression levels of Serpinb3a in acute and diabetic wounds. Immunoblot for Serpinb3a and immunohistochemistry for E-cadherin and Slug was performed on healing skin of immunocompetent Balb/c mice with 5-mm full-thickness splinted wounds at 6 hrs and 2, 4 and 7 days post-wounding. Full-length Serpinb3a was expressed in E. coli and purified to endotoxin-free levels. Serpinb3a identity and inhibitory function was characterized by immunoblot and Cathepsin G assay. Therapeutic efficacy of Serpinb3a was evaluated in 5-mm full-thickness splinted wounds in C57BL6/J and diabetic and obese BKS.Cg-Dock7m +/+ Leprdb/J (db/db) mice. Wound closure was evaluated by planimetry. In vitro closure was measured by HaCaT scratch assay and compared to EGF.
Results: NCBI GEO analysis indicated previously unreported evidence for skin-specific expression of Serpinb3a after dermal wounding, but not mucosal (tongue) wounding ($p<0.001$, N=3/group), and further suggested that Serpinb3a expression was lower in migrating keratinocytes at the wound edge of db/db but not C57BL6/J mice ($p<0.05$, N=3/group). Immunoblot analysis of wounds in Balb/c mice demonstrated high levels of Serpinb3a within 6 hours of wounding, peaking at 2-4 days and resolving by day 7 post-wounding (N=3/timepoint). Protein levels of Serpinb3a correlated tightly with immunohistochemical markers of EMT (E-cadherin loss and nuclear localization of Slug) in wound edge keratinocytes. Recombinant Serpinb3a was topically applied to acute and diabetic wounds. Significant acceleration of early closure, during the epidermal EMT phase of healing, was observed with exogenous Serpinb3a in both C57BL6/J and db/db mice ($p<0.05$, N=2-8). Treatment of HaCaT cells with Serpinb3a significantly accelerated wound closure compared to controls and at levels comparable to EGF ($p<0.01$, N=2 independent experiments).
Conclusion: Serpinb3a is a novel endogenous mediator of wound healing in the skin and represents a potential therapeutic modulator to accelerate poorly healing wounds. Topical or controlled delivery of Serpinb3a may provide a new treatment approach for tissue repair and regeneration.

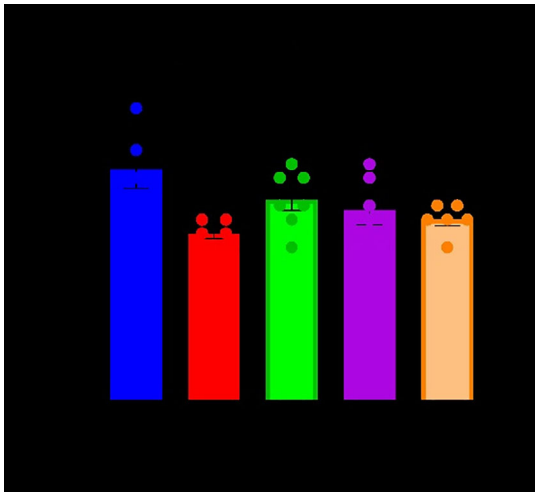
J4.03 | LncRNA Malat1 Modulates Macrophage Polarization By Promoting MIR-155

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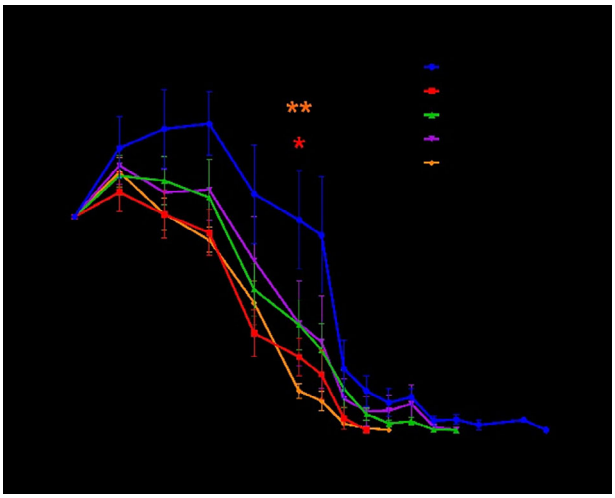
Background: Macrophages play an essential role during wound healing. In response to signals from the surrounding microenvironment, macrophages dynamically adapt between M1 and M2 phenotypes. When macrophages are inappropriately activated, adverse outcomes, such as fibrosis or chronic non-healing wounds, will be the consequence. The underlying molecular mechanisms of macrophage polarization are still unclear. Increasing evidence indicated that non-coding RNAs (microRNAs and lncRNAs) are involved in macrophage polarization. We hypothesize that lncRNA Malat1 plays a role in macrophage polarization through NF-kB/miR-155 signaling pathway.
Methods: Mouse macrophages (RAW264.7 cells) were treated with either LPS or IL-4 to generate M1 and M2 macrophages, respectively. MALAT1 knockdown was achieved by siRNA transfection. Genes expression was analyzed using real-time PCR.
Results: Raw macrophages were polarized to M1 phenotype with the treatment of IFN-r+LPS, and M2 phenotype with the treatment of IL4, which was confirmed with M1 or M2 marker gene expression. Malat1 and miR-155 were highly expressed in M1 macrophage, other than M2 macrophage. Knock-down of Malat1 resulted in significantly decreased production of pro-inflammatory makers including iNOS, IL1-beta, TNF-α, and miR-155, while significantly increased expression of M2 marker genes CD206. Mechanistically, it was revealed that Malat1 modulated macrophage polarization by promoting miR-155 via the NF-kB signaling pathway.
Discussion: These findings provide evidence that Malat1 promotes pro-inflammatory macrophage phenotypes through the cascade of NF-kB/miR-155. Furthermore, these results suggest Malat1 might serve as a molecular regulator in macrophage polarization and is a potential therapeutic target for wound healing.

J4.04 | Topical Delivery Of Cerium Oxide Nanoparticles Conjugated With Microrna-146A Accelerates Diabetic Wound Healing

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Background: Diabetic wounds are a devastating ailment for many diabetic patients with increasing prevalence and morbidity. The complex pathophysiology of the diabetic wound environment has made finding effective treatments difficult. Our lab has previously demonstrated that an intradermal injection of cerium oxide nanoparticles conjugated to microRNA-146a (CNP-miR146a) accelerates diabetic wound healing by modulating inflammation and oxidative stress in diabetic mouse and pig models. The purpose of this study is to test the effectiveness of topical application of CNP-miR146a using a cellulose gel delivery system as opposed to our traditional intradermal injections and then compare our treatment gel to Granexin®, a cellulose gel loaded with ACT1, a peptide mimetic of connexin43 that has undergone clinical testing in diabetic wounds.

Methods: Diabetic mice (db/db) were administered single, 8 mm full thickness punch biopsy wounds on the dorsal skin. Treatments were applied intradermally (PBS, CNP-miR146a) or topically as a gel (cellulose, Granexin®, CNP-miR146a) to the wound area. Wounds were photographed every other day and wound area was calculated using ImageJ.

Results: The average day of full wound closure for CNPmiR146a treated by intradermal injection (Day 12) and as a topical gel (Day 13) were significantly lower compared to PBS treatment (Day 16) (Figure 1). In addition, both CNPmiR146a treatment applications showed improved wound closure rate compared to Granexin® gel and PBS treatments. Wound area on Day 10 was significantly lower for both CNP-miR146a intradermal injection ($p<0.05$) and CNP-miR146a topical gel ($p<0.01$) when compared to PBS. No significant decrease in wound area was found on Day 10 comparing Granexin® gel or cellulose gel treatments to PBS treatment (Figure 2).

Conclusion: Our results demonstrate that a topical CNP-miR146a gel accelerates wound healing in diabetic mouse wounds, with improved wound closure rate compared to the clinically tested Granexin® cellulose gel. This topical CNPmiR146a gel treatment will offer an easy application method for diabetic patients to help improve chronic wound healing.

J4.05 | MiR-2052 Alleviates Hyperglycemia-Induced EMT in Keratinocyte

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Background: Epithelial to mesenchymal transition (EMT) is a critical process that enables cutaneous wound healing. In response to injury, EMT is required to activate and mobilize stationary keratinocytes in the skin toward the wound bed, enabling epithelialization. This process is dysregulated in diabetic wounds. MicroRNAs regulate many biological processes. Specifically, miR-205 influences multiple cellular processes, including EMT. We have previously shown that miR-205 is dysregulated in diabetic wounds. However, the role of miR-205 in diabetic wounds remains undefined. We hypothesized that overexpression of miR-205 would alleviate hyperglycemia-induced EMT in keratinocytes.

Methods: To test our hypothesis, we incubated HaCaT Keratinocytes with media containing 5 mM glucose (low glucose), or 25 mM glucose (high glucose) for 24 hours. The overexpression of miR-205 was achieved by miR-205 mimic transfection. Real-time PCR was performed to evaluate genes expression.

Results: High glucose conditions induced HaCaT cells to undergo an EMT transition, as confirmed by its morphological change to spindle-shaped cells, decreased expression of epithelial marker CDH1, as well as enhanced expression of mesenchymal markers, including CDH2, and SM22. Notably, MiR-205 was remarkably induced by high glucose in HaCaT cells. Overexpression of miR-205 in HaCaT cells significantly reduced mesenchymal makers CDH2 and SM22, while significantly induced epithelial marker CDH1. Mechanistically, miR-205 modulated EMT by downregulating SMAD4, the potent EMT activator in the TGF-beta pathway.

Discussion: These findings suggest that miR-205 may regulate EMT in hyperglycemia conditions as seen in diabetic wounds. The downregulation of miR-205 in diabetic wounds may lead to the dysregulated EMT and slower reepithelialization observed in diabetic wounds. Therefore, miR-205 may represent a potential therapeutic target to address the dysregulated EMT underlying diabetic wound healing impairment.

J4.05 | WITHDRAWN

WHS SESSION K: CONCURRENT ORAL ABSTRACTS II

K1: Mechanoregulation

K1.01 | Allometric Tissue-Scale Forces Activate Mechanoresponsive Immune Cells To Drive Pathological Foreign Body Response To Biomedical Implants

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Barrera[1], Hudson Kussie[1], Noah Magbual[1], Mimi Borrelli[1], Artem A. Trotsyuk[1], Sun Hyung Kwon[1], James Dunn[2], Zeshaan Maan[1], Michael Januszyk[1], Lukas Prantl[3], Geoffrey C. Gurtner[1]

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Background: The longevity of biomedical implants is limited by pathological foreign body response (FBR), leading to implant failure. Nearly 90% of all implant failures are associated with FBR and up to 30% of all implantable devices will undergo failure during their lifetime. Despite the prevalence of FBR-mediated implant failure, the underlying mechanisms are not well understood. Our incomplete understanding of the FBR is exacerbated by the inability of standard laboratory models to recapitulate the highly fibrotic response associated with implant failure in humans. Allometric scaling principles govern inter-species differences in biological properties resulting from changes in the size of the organism and predict that tissue-scale forces increase exponentially as the body size increases. The impact of these allometric tissue-scale forces on the activation of mechanical signaling and its consequences for overall FBR has not been investigated.

Methods: We analyzed a unique, large volume human tissue library consisting of breast implant FBR capsules with different degrees of fibrosis. We analyzed the human tissue specimens using a next generation sequencing-based quantitative assay against 2500+ known biomarkers for inflammation and fibrosis. Further, we modeled the local mechanical stress patterns resulting from tissue-scale forces at the implant-tissue interface in mice and humans using finite element modeling. Next, we developed a mechanically stimulated implant (MSI) model to recapitulate human-like tissue-scale forces in mice. We compared the fibrous capsules formed around standard implants and MSIs in mice using histopathology and single cell sequencing, and compared the results with human implant capsules. Finally, we blocked mechanical signaling in the human-like MSI mouse model and analyzed the resultant FBR using histopathology and immunostaining.

Results: We first demonstrated that pathological (highly fibrotic) FBR in humans is mediated by immune cell-specific Rac2 mechanotransduction signaling, independent of implant chemistry or mechanical properties. We then showed that mice, which are typically poor models of human FBR, can be made to induce a strikingly human-like pathological FBR by altering tissue forces. Altering tissue forces alone activates Rac2 signaling in a unique subpopulation of immune cells and results in a human-like pathological FBR at the molecular, cellular, and local tissue levels. Finally, we demonstrated that blocking Rac2 signaling negates the effect of increased tissue forces, dramatically reducing FBR.

Conclusions: For decades, it has been assumed that the foreign body response to biomedical implants is primarily a reaction to the chemical and mechanical properties of the implant. Here, we show for the first time that a third independent variable, allometric tissue-scale forces (which increase exponentially with body size), can activate mechanoresponsive immune cells and drive the biology of FBR.

K1.02 | Mechanical Regulation Of Provisional Matrix Assembly In 3D Fibrous Microtissues

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Background: During tissue repair, fibroblasts lay down a provisional matrix which serves as a migration base for other cells to close the wound and restore tissue architecture. Although the field has come to appreciate that mechanical forces modulate tissue repair, how tissue mechanics regulate provisional matrix assembly by fibroblasts is poorly understood. To address this problem, we've developed a biomimetic wound-on-chip model wherein fibroblasts ensconced in collagenous matrices close open wounds by assembling a fibronectin-rich provisional matrix and demonstrate that this process is dependent on cellular contractility and extracellular matrix (ECM) alignment of the surrounding tissue.

Methods: A cell suspension of NIH3T3 fibroblasts in collagen type I matrix was seeded in microfabricated polydimethylsiloxane (PDMS) arrays of microwells containing 4 or 8 standing micropillar structures (diameter 100μm, height is 250μm). After tissue compaction around the pillars, microtissues were wounded with a microdissection knife and the tissue's reaction was monitored with time lapse microscopy. To investigate the contribution of fibronectin to the closure process, fluorescently labeled fibronectin was added to the culture medium, or experiments were conducted with fibronectin null cells or under serum free conditions. Actomyosin inhibitors were used to study the role of cell contractility on provisional matrix assembly. To investigate the effect of ECM alignment on provisional matrix assembly, wound closure and provisional matrix assembly was measured in injured rectangular (aligned ECM) versus octagonal (isotropic ECM) microtissues.

Results: Within 24 to 48 hours, 3T3 fibroblasts closed full-thickness wounds in microtissues by assembling a provisional matrix that bridged the gap. Pulse-chase experiments suggested that the provisional template was made of both cellular fibronectin and plasma fibronectin. Tissue closure was abrogated in fibronectin null fibroblasts, but not in microtissues grown under serum free conditions, suggesting that newly synthesized cellular fibronectin was critical for provisional matrix assembly. In presence of contractility inhibitors, the rate of provisional matrix assembly to close the wound was reduced. Intriguingly, the rate of provisional matrix assembly correlated with local ECM alignment of the tissue adjacent to the wound. As such,

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wounds in octagonal tissues closed faster compared to wounds in rectangular microtissues.

Conclusion: Together, these data suggest that provisional matrix assembly is controlled by tissue contractility and alignment of the ECM, which constitutes the mechanical boundary conditions of our model system. A better understanding of how mechanical boundary conditions regulate de novo tissue formation may inform new strategies to promote tissue repair and regeneration.

K1.03 | Mechanical Signaling Mediated by IQGAP1 Promotes Pathologic Foreign Body Response

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Background: Foreign body response (FBR) begins as a wound healing reaction to tissue trauma created from the insertion of a foreign material, such as a biomedical implant. The continued presence of these implants within the body exacerbates this response, promoting the formation of a fibrous capsule around the implant. Over time, this process often leads to implant failure and patient morbidity. The identification of rational therapeutics that inhibit the effects of mechanical stimulation on a cellular level, and therefore reduce the pathologic inflammation present around biomedical implants, represents a promising area of investigation. In this study, we utilized a genomics and proteomics based approach to analyze both murine and human FBR tissue. Our goal was to identify a molecular mediator of mechanical signaling pathways that may ultimately lead to the design of novel precision therapeutics for mitigating pathologic FBR.

Methods: FBR capsules from explanted human biomedical devices, murine FBR capsules, and control human and murine subcutaneous tissue were analyzed using mass spectrometry. Pathway analysis of the top upregulated proteins in human and murine FBR tissue was generated via STRING Functional Enrichment Analysis. To investigate FBR in mice, we employed a murine model of mechanically stimulated implants (MSIs). Finally, we performed single cell RNA sequencing (scRNA seq) of cells isolated from murine FBR capsules and further assessed the FBR capsules using histological and immunohistochemical staining.

Results: Our STRING pathway analysis revealed IQ motif containing GTPase Activating Protein (IQGAP1) as being a central meditator common to several of the top upregulated pathways in both human and murine FBR. In our mouse model of FBR, we found that IQGAP1 deficient mice displayed a significantly reduced FBR as evidenced by thinner capsules, lower levels of collagen deposition, collagen maturity, and myofibroblast activation. Our scRNA seq analysis revealed that IQGAP1 deficiency resulted in diminished transcription of mechanotransduction (Rac1), inflammation (Ccl3, Ccl4, Il1b), and fibrosis related (Acta2, Cxcl2, Cxcl5, Ptx3) genes, which was confirmed on the protein level with immunofluorescent staining.

Conclusion: Collectively, our results demonstrate that targeting mechanical signaling at the implant tissue interface may improve the biocompatibility of biomedical implants, with broad implications for clinical application. We investigated a potential molecular target involved in pathologic FBR around biomedical implants and showed that genetic deficiency of IQGAP1 mitigates mechanically stimulated FBR by diminishing downstream mechanical, inflammatory, and fibrotic pathways. Therefore, IQGAP1 may be a promising molecular target for rational therapeutic design to mitigate pathologic FBR.

K1.04 | Wound Stratification Elucidates Novel Genomic Persepctive Of Negative Pressure Therapy In A Porcine Model

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Statement of Purpose: Negative Pressure Wound Therapy (NPWT) has been shown to modulate the behavior of wound tissue. Yet, the dynamic mechanism(s) of how NPWT exerts its effects at a molecular and cellular level remains unresolved; specifically, the key early signaling pathways initiated and the roles of different cellular populations upon exposure to negative pressure and different dressing materials. To date, wound analyses have only evaluated gene expressional profiles of whole wound isolates. In this study we provide a novel perspective that compares the expressional profile of "Full" wounds relative to each independent layer of wounded skin (epidermis vs dermis vs subcutaneous). Additionally, we investigate how different dressing materials augment the early wound environment in the setting of NPWT.

Methodology: An acute incisional porcine wound model was developed by inflicting 2cm (long) x 2cm (deep) incisions on the flanks of two female Yucatan pigs (n=2). Four different wound dressings were applied within the incisional wounds, in addition to one control group. Dressing materials included Owen's Rayon™, Granufoam™, Polycaprolactone (PCL) Mesh, and Fibrin Glue. Wounds were then treated with or without NPWT and evaluated over 8 hours. Tissue samples were collected at 0-, 2-, 4-, and 8-hours post-wounding and processed for RNA sequencing. Specimens, transverse to the incision, were bisected along the incision, with one side representing "Full" wound samples and the opposing side further dissected into the epidermis, dermis, and subcutaneous layers.

Results: RNA sequencing demonstrated substantial differences in expressional profiles between individual layers relative to each other and between each independent layer relative to the "Full" wounds.

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Localization of expressional changes in key wound signaling pathways to specific layers was also observed, including angiogenesis, inflammation, proliferation, and remodeling. Lastly, dressing material and/or NPWT demonstrated the capacity to modulate early wound signaling.

Conclusion: This study provides valuable information on medical material interactions with wound tissue and/or NPWT and provides insight into early genomic changes of wound tissue. Together this experimental data offers a new perspective and methodology for characterizing wound tissue responses and provides new insight into localizing expressional patterns and genomic profiles within different wound regions to ultimately help improve clinical therapies.

K1.05 | Determining How Early Disruption Of Mechanotransduction Affects Acute Wound Healing

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Background: Previous work has determined that focal adhesion kinase inhibitor (FAKI) can mitigate mechanotransduction-mediated fibrosis and inflammation after 14 days of treatment. We sought to determine how targeted inhibition of FAK affected incisional wound healing during acute time periods.

Methods: A 3 cm incision was made on the mouse dorsum and sutured closed. After 4 days, we attached a mechanical strain device to the dorsum to apply mechanical strain across the wound to create hypertrophic scar (HTS) formation. Of a total of 18 mice, (n=2) received an incision but no device (control); (n=10) received an incision with mechanical strain (HTS); and (n=6) were treated with FAKI hydrogels in conjunction with mechanical strain (S+FAKI). After 3 days of treatment, the mice were sacrificed. These groups and unwounded skin were compared by measuring scar width, analyzing collagen ultrastructure using picrosirius staining and collagen quantification algorithms, and immunofluorescent staining of a TGFB1 dependent fibrotic marker Thrombospondin1 (THBS1).

Results: The average width of the HTS was significantly greater than the width of both the FAKI treated and control scars. The HTS collagen fibers were also significantly longer and more highly aligned than FAKI treated and control scars, as well as unwounded skin. The scars in the HTS mice also had significantly elevated levels of a pro-fibrotic extracellular matrix marker Thrombospondin1 compared to FAKI treated and control scars.

Conclusion: Acute FAKI therapy effectively narrowed scar widths and promoted regenerative collagen architecture formation. Scar formation has been previously found to be TGFB1 dependent, and we measured extracellular matrix marker Thrombospondin1 as a downstream surrogate for TGFB1 expression. Thus, early FAKI could promote healing through the FAK-->TGFB1-->Thrombospondin1 signal pathway. Further understanding of targeting and timing of FAKI therapy can prove important for future therapies.

K1.06 | Comparison Of Fibroblast Versus Macrophage Specific Mechanotransduction Knockout In A Wound Healing Model

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Background: For an injury to heal, a variety of cell types are recruited to begin the healing process. To better understand the impact of certain cell types and their influence on healing, we investigated how fibroblasts and macrophages responded to mechanical signals to influence healing, fibrosis, and regeneration.

Methods: In order to simulate human-like hypertrophic scarring (HTS), we utilized an HTS device that applies mechanical strain on the incisional wounds of mice. These devices are steel palatal expanders with rod extender arms that are attached to the backs of the mice using skin adhesive and staples to allow for a uniform strain profile. We waited 4 days after the incision to mount the device, allowing the wound to close. Next, we extended the device every other day for a 14 day time period. To analyze the scar tissue, we took photos of the scars before we explanted. Then, once we explanted the scar tissue, we stained it using picro-sirius red, a type of staining that magnifies collagen formation and structure. With the stained tissue, we were able to use CurveAlign and CT-Fire collagen architecture algorithms. CurveAlign quantifies all fiber angles within the given image, while CT-Fire analyzes individual fiber measurements. We specifically bred mice to knockout FAK signaling in either fibroblasts (Col1a2 Cre, Ptk2-flox) or myeloid cells (Lyz2-Cre, Ptk2-flox) and ran both breeds of mice through the HTS experiment explained above.

Results: Utilizing these devices created scars in wildtype mice with increased levels of fibrotic tissue and highly aligned collagen as compared to the control wildtype mice without extension. With the photos, we took measurements of both length and width of the scar to compare the fibroblast and myeloid FAK KO scars with wildtype scars. We observed that both knockout strains generated visually less scarring than in wildtype mice. Utilizing the software programs, we analyzed collagen fiber alignment and length. Both of the knockout strains had scar tissue with significantly less aligned, smaller fibers contrasting with the highly aligned, long fibers of wild type HTS mice scar tissue. With these measurements, we are able to demonstrate that the blocking fibroblast or macrophage mechanotransduction almost completely eliminated scar formation, decreased collagen alignment, and promoted skin regeneration.

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Conclusions: Our findings point toward the importance of myeloid cell mechanotransduction signaling during healing, possibly opening new doors for therapeutic treatment of fibrosis.

K2: Platform Technologies in Wound Healing

K2.01 | A Medium Throughput 3D Wound-On-A-Chip Screening Platform To Assess Provisional Matrix Assembly During Stromal Wound Closure

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Background: Provisional matrix assembly is a crucial event during healing of full-thickness wounds and involves dynamic interactions between the fibroblast populations in the wound bed and the extracellular matrix. In previous work, we have demonstrated that this process can be recapitulated in engineered collagenous microtissues in vitro.1 In this work, we describe the development of a 3D wound-on-a-chip screening platform to assess provisional matrix assembly during wound closure in a 96-well format.

Methods: 2-photon polymerization (2PP) 3D printing was used to create a master mold of a micropillar device consisting of 4 micropillars of 150 μm diameter with spherical caps of diameter 200 μm at a height of 350 μm. A polydimethylsiloxane (PDMS) replicate of the master mold was inserted in a stereolithography (SLA) 3D printed holder for casting PDMS micropillar devices in a 96-well plate. After curing and sterilization of the plate, cells seeded in a collagen type I matrix and were allowed to self-assemble into microtissues (1.0 mm X 0.6 mm) anchored to these micropillars. A pulsed nanosecond laser was used to ablate full-thickness wounds with a diameter of 100 μm to 200 μm. To demonstrate the utility of the medium-throughput platform, we optimized seeding density of three different types of fibroblasts (neonatal and adult human dermal fibroblasts (NHDF-neo and NHDF-Ad), and cardiac fibroblasts (CF)) to make microtissues and assessed gap closure area and rate using time-lapse microscopy. To characterize the ECM of the provisional matrix, injured microtissues were fixed and stained for collagen I, collagen III, collagen IV, and fibronectin 24h after injury.

Results: The optimal cell seeding density for forming stable microtissues in a collagen hydrogel of 2.2 mg/mL was 7500 cells/well. Upon injury, CFs (N=2, n=12) closed the wounds in 11.6 ± 0.48 hours, NHDFneo (N=2, n=7) in 26.2 ± 3.53 hours and NHDF-Ad (N=2, n=15) in 59.8 ± 22.05 hours. All three cell types closed the gap by depositing a provisional matrix that contained fibronectin, collagen I, III and IV. Interestingly, based on fluorescent intensity, the levels of fibronectin in the provisional matrix produced by CF were higher than NHDF-neo, which were higher than NHDF-Ad.

Conclusion: We successfully developed a medium throughput screening platform for assessing provisional matrix assembly during stromal wound closure and show that fibroblasts of different tissue origin have different closure rates, which correlates with the expression of fibronectin in the provisional matrix.

References: Sakar, M. S. et al. Nat. Commun. 7, 1–8 (2016).

K2.02 | A Non-Contact Device For Fast Screening Of Infections In Diabetic Foot Ulcer And Venous Leg Ulcer

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Purpose: Infection is the most likely single cause of delayed healing in chronic wounds. Recent study shows that infected wounds have elevated leukocyte esterase activities as compared with non-infected wounds as determined by a portable device - Detec® Esterase. Here we investigated whether the same device, Detec® Esterase, can be used to screen and diagnose the infection of diabetic foot ulcer (DFU) and venous leg ulcer (VLU) at point-of-care.

Method: Wound dressings from 71 DFU patients and 31 VLU patients 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: For DFU patients, the results show that DETEC® Esterase device had good sensitivity (89.8%), fair specificity (45.5%), moderate accuracy (76.1%), and the increase in post-test risk of infection with positive test result of 9.56%. As for VLU patients, the results show that DETEC® Esterase device had comparably good sensitivity (82.1%), moderate specificity (66.7%), good accuracy (80.6%), and a smaller increase in post-test risk of infection with a positive test result of 5.51% because of high prevalence of the condition. Through fisher's exact test to compare the diagnosis efficiency between DFU and VLU, two tailed p-values for sensitivity, specificity, and accuracy are 0.4825, 0.593, and 0.7975, respectively. Therefore, there are no significant differences in the device diagnosis efficiency between two different wounds. Using a logistic regression model, we determined whether other factors (such as age, gender, race, initial wound size, wound location, and sign of infection) would affect the accuracy of DETEC® Esterase device on diagnosis of infection and found none of

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these factors has significant effect on diagnosis accuracy of DETEC® Esterase device (age, P=0.4597; gender, P=0.1886; race, P=02401; initial wound size, P=0.9020; wound location, P=0.8549; signs of infection, P=0.456).

Conclusion: Our results support that DETEC® esterase can effectively and accurately diagnose and/or screen for infections in DFUs and VLUs. The diagnosis accuracy of the device is not influenced by the patients' age, gender, race, initial wound size, wound location, and sign of infection.

K2.03 | Pullulan-Collagen Hydrogel Wound Dressing Promotes Dermal Remodeling and Healing in an Excisional Wound Model

Melissa C. Leeolou, Dharshan Sivaraj, Michael Davitt, Dominic Henn, Sydney Steele, Savana L. Huskins, Artem A. Trotsyuk, Hudson C. Kussie, Autumn Greco, David Perrault, Jagannath Padmanabhan, Michael T. Longaker, Kellen Chen, Geoffrey C. Gurtner

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Background: The fundamental objectives of wound healing therapies are to provide protection against external factors and to sustain optimal moisture levels within the wound bed. Biological scaffolds such as hydrogels provide an ideal, physio-mimetic of native ECM that can improve wound healing outcomes after cutaneous injury. While most studies have focused on the benefits of hydrogels in accelerating wound healing, there is minimal data directly comparing different hydrogel material compositions.

Methods: In this study, we utilized a splinted excisional wound model that recapitulates human-like wound healing in mice and treated wounds with three different collagen hydrogel dressings. The first dressing was composed of 90% collagen and 10% alginate. The second dressing was composed of 55% collagen and 44% cellulose. Finally, the third dressing was composed of 5% collagen and 95% pullulan. We assessed the feasibility of applying each dressing during standard dressing changes and took photographs of the wounds over time to determine the rate of wound closure. We then performed histologic and histopathologic analysis on the explanted scar tissues to assess the effects each hydrogel dressing on collagen architecture and alignment, as well as tissue response.

Results: Wound area changes were measured by analyzing digital photographs. At PODs 10 and 12, the wound area was significantly smaller in wounds treated with the collagen-pullulan hydrogel compared to control wounds. Quantitative analysis of collagen architecture demonstrated that collagen-pullulan treated wounds had a lower amount of mature collagen within the healed scar. Untreated murine wounds showed a trend towards higher collagen fiber alignment, measured by the angle skewness of the fibers. Collagen-pullulan treated wounds showed significantly more random tissue alignment compared to collagen-cellulose treated wounds. Further, collagen-pullulan treated wounds demonstrated significantly shorter tissue fiber length compared to the other wound groups. Collectively, collagen-pullulan hydrogel treatment promoted restoration of a more normal skin phenotype with a basket-weave collagen architecture.

Conclusion: Our data indicate that the material compositions of hydrogel dressings can significantly influence healing time, cellular response, and resulting architecture of healed scars. Collagen-pullulan hydrogel therapy accelerated wound closure and promoted healed tissue with shorter, less dense, and more randomly aligned collagen fibers, compared to other dressings. Further understanding of how specific hydrogel properties affect the healing and resulting tissue architecture of wounds may lead to novel insights and further optimization of the material properties of wound dressings.

K2.04 | Toward Immunocompetent, Vascularized, Autologous 3D Skin Model Reconstructed By Tissue Engineering For Wound Healing Studies

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Skin wound healing is a process occurring after injury and is categorized into four phases: hemostasis, inflammation, proliferation, and remodeling. The early phases require the recruitment of several immune cells from the blood that regulate the fate of wound healing. However, the role of skin's resident immune cells is not well defined. They could perpetuate a distinct role in the process compared to circulating immune cells. To investigate their contribution to wound healing, we first developed a cell extraction technique that isolates skin resident cells from the same donor (keratinocytes, fibroblasts, immune and endothelial cells). Analysis by flow cytometry reveals the presence of resident skin macrophages CD45+ CD14+; lymphocytes, CD45+CD3+; dendritic cells CD45+, CD14-, CD1a+ and endothelial cells CD45-CD31+ in freshly isolated skin cells. From this isolation, an autologous three-dimensional (3D) human skin model was developed in vitro by self-assembled method. Their presence was then confirmed in the 3D skin model by immunofluorescence staining. Endothelial cells self-assemble to form capillary like networks. The number of immune cells is constant after 14 days in culture and cells were able to produce TNF-α cytokine. The rate of proliferation at basal layer of the epidermis

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characterized by Ki67 staining, reveal an important contribution of immune and endothelial cells in reconstructed skin (N=4). This complete and autologous skin model provides a great tool to study the interaction of skin's dermal resident immune cells in the context of wound healing.

K2.05 | Real-Time Measurement And Tracking Of Wound Healing

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The goal of this study was to evaluate the use of a new smart phone imaging technique to assess healing of skin wounds at the point-of-care in real time.

Initially photos of a wound were collected after each dressing change (every 3 to 4 days). Photoshop was used to isolate and standardize the wound. For the purposes of this analysis, 'wounded' areas were defined as light-red granulation tissue while 'unwounded areas' were defined as everything else. The wounded areas were masked in black and the intact areas were electronically removed leaving a white background. This produced a black and white image of the wound. A Python script was written to calculate the proportion of black pixels and white pixels of the image to determine the relative proportion of damaged to undamaged/healed areas. The time to time rate of change in the wound size, or healing rate, is analogous to the rate at which the black pixels vanish. The data generated was very useful but was not point-of-care. To convert this to a point-of-care wound healing tool the Python script was converted to Swift and C++ language platforms for processing on iPhones. The new program allowed the smart phone to use visual analysis algorithms and the RGB (Red Green Blue) coordinates of the wound to map out the wound surface and mask it. The software then calculated the change in wound size since the last reading. The system was used to follow the healing of a deep wound with a missing flap and exposed 10cm of canon bone in a horse. The results were plotted as rate of healing over time and as percentage change in wound size between dressing changes.

The initial work with Photoshop showed that the wound area could be mapped and followed using software. The second stage showed that the wound surface area calculated by the new algorithms was within 1% of the surface area calculated by Photoshop. Asymmetry of the wound or its healing process did not cause any problems in the assessment.

Using a visual analyses algorithm and a smart phone, it has been demonstrated that mapping the entire wound healing process could be done at the point-of-care. This will greatly facilitate clinical care.

K2.06 | WITHDRAWN

K3: Skin Infection, Inflammation and Innate Immunity

K3.01 | Activation Of Lasr And Specific Enzyme Defense Genes Results In Pseudomonas Aeruginosa Changing From Plancktonic To Biofilm Forming

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Pseudomonas aeruginosa (PA) is an opportunistic pathogen frequently isolated from cutaneous chronic wounds. Certain PA strains are highly virulent, establish strong biofilm and are antibiotic resistant. PA growth is regulated by the quorum sensing system lasR to manage virulence and response to high oxidative stress (OS) generated by phagocytes during inflammation. How PA forms biofilm in chronic wounds in response to OS is still unknown. We hypothesize that PA in the microbiome of the skin becomes biofilm-forming in chronic wounds when high levels of OS activate lasR, and catalase (katA and katB) and superoxide dismutase (sodA and sodB), enzymes that break down H2O2 released by neutrophils in the wound into water and oxygen. To test this possibility, we used a biofilm-forming PA strain isolated from our murine chronic wounds and measured the transcriptional response of PA in the presence and absence of H2O2-induced OS. RNA was extracted, treated with RNAlater overnight and reversed transcribed into cDNA for quantitative polymerase chain reaction. We show that PA in mature biofilm, when compared to planktonic cells in log phase, has significantly increased basal expressions of lasR, sodA, sodB and katB (log2FC=13.22, 7.43, 10.67, and 7.36, respectively, $p<0.05$). After 4hr of H2O2 treatment, expressions of lasR, sodA, katA and katB increases (log2FC=16.03, 13.65, 12.58 and 13.50, respectively), showing that PA in biofilm respond to OS readily compared to planktonic cells. This is clearly shown when planktonic cells, treated with H2O2 are compared to untreated cells, respond poorly to OS as shown by significantly decreased expressions of lasR, sodA, katA and katB (log2FC=-0.04, -3.97, -5.54 and -4.03, respectively). Taken together, these results indicate that PA in biofilm has the advantage of withstanding OS through the proper activation of the lasR quorum sensing system, and catalase and superoxide dismutase genes, two major antioxidant systems. In conclusion, PA survives the harsh, high OS microenvironment present in chronic wound and colonizes these wounds with biofilm by turning on specific survival genes. Therefore, effective biofilm removal may be accomplished by disrupting lasR sodA, katA and katB expression. Because it is well known that biofilm in human chronic wounds readily returns after debridement, these finding could have major implications for treatment of human chronic wounds immediately after debridement.

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K3.02 | Wound Macrophage-Derived Oncostatin M Induces Antimicrobial S[1]00a9 In Cutaneous Wound Epithelium

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Background. Wound macrophages play multifaceted role in cutaneous wound healing. Emergent line of evidence argues in favor of a strong macrophage-keratinocyte cross-talk. This work builds on previous observation that human wound-site macrophages and wound fluid is rich in Oncostatin M (OSM). OSM, unlike other macrophage-derived cytokines exclusively involved in inflammation, primarily functions by its effect on keratinocytes. A diverse and networked antimicrobial defense system in keratinocytes defends against skin infections. Antimicrobial peptides/proteins (AMPs) like β-defensins and S100 proteins (S100A7, S100A8, and S100A9) represent a key innate defense mechanism of the human skin. We sought to test the significance of OSM on antimicrobial defense system of the cutaneous wound epithelium (WE).

Methods and Results. Laser capture microdissection and proteomics studies identified S100A9 as being highly expressed in the human chronic cutaneous WE. Importantly, OSM present in human chronic wound fluids potently induced ($p<0.001$; $n = 4$) the expression of S100A9 in human keratinocytes. In addition, OSM induced β-defensins, establishing a cause and effect link between wound-site OSM and keratinocyte AMPs. OSM repressed keratinocyte SOCS3, a negative regulator of JAK-STAT signaling. In turn, in keratinocytes with repressed SOCS3, OSM-induced S100A9 was potentiated. OSM receptor knockdown abrogated ($p<0.05$; $n = 4$) OSM-induced S100A9 expression suggesting that OSM mediated suppression of SOCS3 drives JAK-STAT signaling leading to S100A9 induction. S100A9 over-expression in human keratinocytes induced ($p<0.05$; $n = 4$) the expression of specific β-defensins and increased the killing of S. aureus. The ability to fight S. aureus infection was compromised ($p<0.05$; $n = 4$) in S100A9-null mice. Finally, wound fluid from human chronic wounds with poor infection outcomes was associated with low ($p<0.05$; $n = 7$) OSM and S100A9 in wound fluid.

Conclusion. Taken together, this work establishes the critical significance of wound macrophage-keratinocyte cross-talk in endowing the WE with antimicrobial defenses. Wound macrophage derived OSM enables such cross-talk adding a new dimension to the significance of wound inflammation.

K3.03 | RNA-Seq Analysis Displays Divergent Early Gene Expression Profiles In Aureus Infecting The Wounds Of Wild Type Compared To DB/DB Mice

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Purpose: Since skin wounds compromise the epidermal barrier, development of infections is always a possibility. For delayed healing wounds in particular, such as those that tend to occur in diabetic or otherwise healing-impaired patients, the threat of wound colonization by infectious microorganisms, such as Staphylococcus aureus, is of particular concern. The ways in which bacteria adapt to the wound environment, and how these adaptation processes differ in healing-impaired wounds, are not fully understood.

Methods: Here we used a murine full-thickness splinted excisional wound model infected with S. aureus (UAMS-1 strain) in both wild type (WT) C57BL/6 mice and diabetic model db/db mice. At post-infection day 3 and day 7 we harvested bacterial RNA from the wound bed and performed bulk RNA-seq to compare gene expression profiles. We also compared these gene expression profiles to those of planktonic S. aureus cultured in vitro.

Results: Mapping to the S. aureus and Mus musculus genome revealed that, in addition to S. aureus RNA, samples also contained murine RNA, which could be quantified and excluded based on mapping to the constructed genomes. Differential analysis of S. aureus genes revealed dramatically different gene expression profiles at post-infection day 3 between bacteria from WT and db/db mice. The majority of this difference resolved by post-infection day 7, though a number of differences remained. All samples harvested from wound-infecting S. aureus revealed dramatic differences in gene expression from in vitro cultured S. aureus, irrespective of time point or host mouse genotype.

Conclusions: These data suggest that the early wound environment in WT and db/db mice drives dramatically different gene expression paradigms, and that some aspects of this differential expression are retained until later in the wound healing process. Future experiments will seek to characterize the host organism responses that accompany these variable expression paradigms in the hopes of better understanding the characteristics of the wound environment that yield differential.

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K3.04 | Cerium Nitrate Protects Skin Models From Bacterial Penetration

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Background: Infection is the leading cause of mortality among burn patients and responsible for up to 50% of burn-related deaths. Multiple topical products have been developed to control burn infections to improve the healing. Among wound care products, Silver Sulfadiazine cream (SSD) is the standard of care in the US for the management of deep burn wounds. In contrast, in Europe and South America, for this purpose SSD cream combined with cerium nitrate (CeN), Flammacerium, has been widely used. Compared to SSD cream, CeN+SSD cream has been recognized by burn surgeons to have the following benefits: hardening of the eschar resulting in less bacterial colonization, infection, and invasion. To investigate these recognized benefits of CeN, we studied Pseudomonas penetration into skin models (in vitro 3-D collagen model and an ex-vivo porcine burn skin model), and the potential mechanism by which CeN can protect against penetration.

Methods: 3-D collagen matrix (rat tail collagen) or porcine skin was treated with CeN (40 mM) or water (control) for 30 min. In the collagen matrix, mechanical changes due to these treatments were measured by rheometry, and the penetration of P. aeruginosa was visualized by confocal microscopy. In the burned porcine skin model, the penetration of P. aeruginosa was assessed by recovering the bacteria on a filter membrane (0.2 μm) underlying the skin. Lastly, Pseudomonas-inoculated burned porcine skin was treated with silver dressing and viable counts were determined.

Results: CeN treatment caused the 3-D collagen matrix to become more brittle, characterized by increased stiffness and reduced yield strength. In the deeper layers of the inoculated CeN-treated 3-D collagen gel, compared to water-treated collagen gel, fewer P. aeruginosa were present. Similarly, CeN-treatment resulted in fewer P. aeruginosa invading the burned porcine skin. Lastly, fewer Pseudomonas counts were found in the burned porcine skin following treatment with silver dressing

Conclusions: CeN treatment resulted in less Pseudomonas penetration and fewer viable counts in the burned porcine skin after treatment with silver dressing.

K3.05 | Filamentous Bacteriophage Delay Healing Of Pseudomonas-Infected Wounds

Arya Khosravi[1], Michelle S. Bach[1], Christiaan R. de Vries[1], Johanna M. Sweere[1], Medeea Popescu[1], Qingquan Chen[1], Aviv Hargil[2], Jonas D. Van Belleghem[2], Gernot Kaber[2], Elizabeth B. Burgener[3], dan Liu[2], Quynh-Lam Tran[2], Tejas Dharmaraj[1], Vivekananda Sunkari[1], Swathi Balaji[4], Nandini Ghosh[5], Shomita S. Mathew-Steiner[5], Sundeep G. Keswani[4], Niaz Banaei[6], Laurence Nedelec[1], Chandan K. Sen[5], Venita Chandra7, Patrick R. Secor8, Gina Suh9, Paul Bollyky[1]

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We have identified a novel role for Pf, a filamentous bacteriophage (phage), in the delayed healing associated with chronic Pseudomonas aeruginosa (Pa) wound infections. Pf is prevalent in chronic Pa wound infections and may promote disease by contributing to biofilm formation and antibiotic resistance as well as directly impairing host immune defenses. We now demonstrate that Pf directly delays wound re-epithelialization by impeding epithelial migration. Utilizing murine and porcine models of wound infection, we reveal Pf impairs healing and epithelial barrier integrity of wounds infected with Pa, as determined by histology, morphometric analysis, and transepidermal water loss studies (TWELS). Further, Pf is able to inhibit healing even in the absence of live Pa, indicative that Pf promotes chronic wounds independent of Pa pathogenesis. Mechanistically, Pf phage directly inhibits autocrine signaling of CXCL1 (KC) resulting in to keratinocyte migration and wound closure. In agreement with these studies, a prospective cohort study of 36 human patients with chronic Pa wound infections revealed that wounds infected with Pf positive strains of Pa took longer to heal and were more likely to increase in size compared to wounds infected with Pf negative strains. Together, these data implicate Pf phage in the delayed wound healing associated with Pa infection through direct manipulation of mammalian target cells. We propose that Pf phage may have potential as a biomarker and therapeutic target in delayed wound healing.

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K3.06 | Low Abundance Of Microrna-21 In Diabetic Wound Macrophages Compromises Resolution Of Inflammation

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Background. Unresolved inflammation impairs diabetic wound healing. Previous work by our laboratory have demonstrated decreased clearance of apoptotic cells (efferocytosis) by diabetic wound macrophages that compromises timely resolution of wound inflammation. Interestingly, we also observed that delivery of miR-21 to human monocyte-derived macrophages (MDM) bolsters efferocytosis resulting in a net anti-inflammatory phenotype. Thus we hypothesized that diabetic wound macrophages have lower abundance of miR-21 that leads to persistent inflammation.

Method. To address our hypothesis, wound macrophages were isolated from well-developed negative-pressure wound therapy (NPWT) dressings (for human) and subcutaneously implanted PVA sponges (for mice). Results. Both human and murine diabetic wound macrophages showed significantly lower miR-21 levels compared to non-diabetics ($p<0.05$; $n=6$). To determine the significance of lower miR-21 in wound macrophages fate and resolution of inflammation, a myeloid cell-specific miR-21 knockdown mice (miR-21/LysM) was developed in our laboratory by crossbreeding mice carrying floxed miR-21 allele (miR-21fl/fl) with LysM-Cre mice. Expression of pro-inflammatory markers in miR-21/LysM wound macrophages were significantly higher while those of reparative phenotype was attenuated ($p<0.05$; $n=6$). Wounds of miR-21/LysM animals suffered from increased neutrophil abundance and persistent inflammation and impairment in wound closure ($p<0.05$; $n=6$). Bioinformatics analysis predicted the neutrophil chemoattractant GRO-α to be a direct target of miR-21. Using 3'-UTR firefly luciferase reporter, GRO-α was discovered to be a direct target of miR-21 ($p<0.05$; $n=6$). Lower miR-21 in diabetic and miR-21/LysM wound macrophages desilenced GRO-α ($p<0.05$; $n=6$) resulting in increased abundance of neutrophils at the wound-edge tissue. Delivery of nanoparticle-based delivery of miR-21 to diabetic wound-site macrophages resolved inflammation and accelerated wound closure ($p<0.05$; $n=6$).

Conclusion. This study directly implicates a miR-21-GRO-α pathway responsible for compromised resolution of inflammation in diabetic wounds.

K4: Dressings and Therapeutics

K4.01 | Myo-Inositol In Fermented Sugar Matrix Improves Human Macrophage Function

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Background: Reactive oxygen species production by innate immune cells plays a central role in host defense against invading pathogens at wound-site. A weakened hos-defense may result in persistent infection leading to wound chronicity. Fermented Papaya Preparation (FPP), a complex sugar matrix, bolstered respiratory burst activity and improved wound healing outcomes in chronic wound patients. The objective of the current study was to identify underlying molecular factor/s responsible for augmenting macrophage host defense mechanisms following FPP supplementation.

Methods & Results: In depth LC-MS/MS analysis of cells supplemented with FPP led to identification of myo-inositol as a key determinant of FPP activity towards improving macrophage function. Myo-inositol, in quantities that is present in FPP, significantly improved macrophage respiratory burst and phagocytosis via de novo synthesis pathway of ISYNA1. Additionally, myo-inositol transporters, HMIT and SMIT1, played a significant role in such activity. Blocking these pathways using siRNA attenuated FPP-induced improved macrophage host defense activities. FPP supplementation emerges as a novel approach to increase intracellular myo-inositol levels. Such supplementation also modified wound microenvironment in chronic wound patients to augment myo-inositol levels in wound fluid (clinicaltrials.gov; NCT02332993).

Conclusion: These observations indicate that myo-inositol in FPP influences multiple aspects of macrophage function critical for host defense against invading pathogens.

K4.02 | Thermal Burn Injury To A Rabbit Ear Shows Decreased Scar Hypertrophy Following Topical Application Of Amiloride

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Purpose: Thermal injury wounds produce skin disfigurement in the form of hypertrophic scars. Victims of severe contact thermal injuries, like deep dermal burns, require an array of treatments to prevent infection, dehydration, and metabolic instability. Although treatments that promote adequate wound healing are critical, limited pharmacological interventions exist that target the resulting scars.

Methods: In this study, we induced 1-cm-diameter contact burn wounds onto the ears of rabbits and analyzed scars following topical application of either amiloride, celecoxib or a control vehicle. We harvested the scars at post-burn day 29 and analyzed tissues for scar elevation index and gene expression.

Results: The results showed that topical amiloride, when applied after wound reepithelization, can significantly reduce the scar elevation index (SEI) of burn-induced scars. Although Celecoxib also showed a reduction in SEI, this decrease was not statistically significant. Tissue from amiloride-treated scars were also analyzed for expression of PTGS2, ACTA2 and COL1A1, but there were no significant

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differences between the scars of vehicle control-treated and drug-treated wounds.

Conclusions: Our results demonstrate that topical administration of amiloride was sufficient to reduce scar elevation, an important clinical indicator of scar severity. Future experiments are required to better understand the mechanisms underlying amiloride-induced antagonism of scar hypertrophy.

K4.03 | A Novel Hyaluronic Acid Matrix Quells The Inflammatory Phase Through The Activation Of NFKB, TNF-A And Arg1 To Accelerate Wound Healing In A Diabetic Mouse Preclinical Model

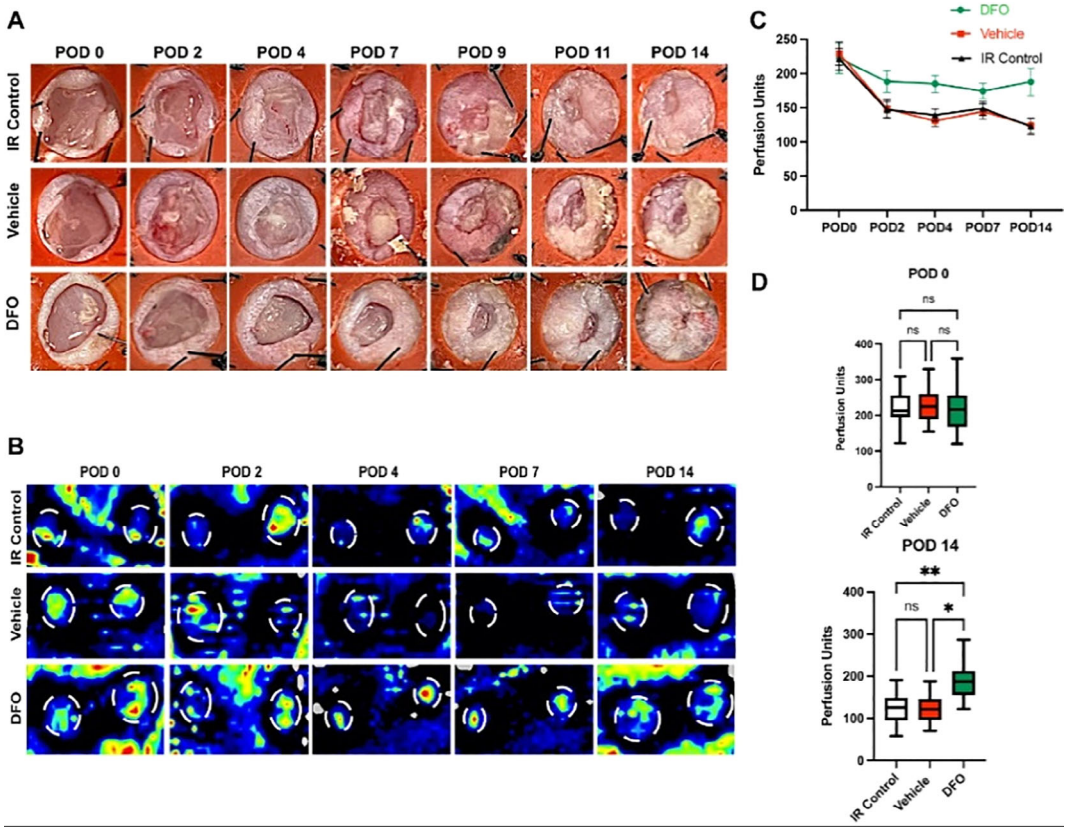
Mitchell Sanders[1], Da Wei[1], Lindsay Polan[1], Mia Hanna[1], Nicole Siegmund[1], ted bundrick[2], Thomas E. Serena[3]

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In normal wound healing, fibroblasts produce hyaluronic acid (HA) which is an important extracellular matrix component required for tissue repair and regeneration by driving cell proliferation, protecting growth factors and stem cells, and preventing infection through bacteriostasis. However, in a diabetic wound, fibroblasts are senescent and as a consequence are often stalled in the inflammatory and proliferative phases of wound healing. We utilized a diabetic mouse wound healing model to demonstrate that a novel hyaluronic acid matrix derived from American Alligator (aHA) can accelerate wound healing through the gene activation of important biomarkers that quell the inflammatory phase and stimulate wound healing. Immunohistochemistry and qPCR indicate that the novel aHA matrix activates the important transcription factor NF-kB which plays a pivotal role in mediating the inflammatory response with a 2.6 fold increase in NF-kB on day 3, concomitant with the upregulation of TNF-a (1.7 fold) and an activation of M2A macrophages based on Arg 1 gene expression and immunofluorescence staining. Our recent findings indicate that replenishing a diabetic wound with aHA will accelerate wound healing by quelling the inflammatory phase and activating M2A macrophages to jump start the cell proliferation. These findings have profound clinical implications for restarting chronic wound healing prior to using more advanced and expensive therapies such as NPWT or human allografts.

K4.04 | Transdermal Deferoxamine Enhances Wound Healing In Chronically Irradiated Skin In Mice

Hendrik Lintel, Darren B. Abbas, Christopher V. Lavin, Michelle Griffin, Nicholas Guardino, Jason L. Guo, Amanda F. Spielman, Geoffrey C. Gurtner, Michael T. Longaker, Derrick Wan



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Background: Radiation-induced fibrosis is a common chronic sequelae of radiation therapy. Chronic radiation dermatitis is characterized by a hypovascular dermis and a fibrotic state which presents many clinical challenges to healthcare providers, including impaired wound healing. Transdermal deferoxamine (DFO), an iron chelator, has been shown to mitigate radiation-induced skin fibrosis in mice through improved angiogenesis and a reduction in reactive oxygen species production. In this study, we aim to investigate the effects of DFO on excisional wound healing in chronically irradiated skin in a mouse model.

Methods: Thirty C57BL/6 mice had their dorsal skin irradiated with 30 Gy fractionated over 6 doses of 5 Gy every other day. After waiting 4 weeks post-radiation to allow for the development of chronic fibrosis, two 5mm excisional wounds were created and stented open on the irradiated dorsum of each mouse. The mice were equally divided into 3 groups: an irradiated (IR) control, a vehicle-only patch, and a 1 mg/cm2 DFO-loaded patch. Every two days, patches and dressings were changed, wounds were photographed, and wound perfusion was assessed via Laser doppler until wound closure. Data analysis was done via one-way ANOVA to compare means between the treatment groups with statistical significance determined at $p<0.05$.

Results: DFO-treated mice grossly demonstrated faster wound closure rates than the vehicle and IR control groups (A). Laser doppler analysis also revealed increased perfusion in the DFO-treated wounds throughout the wound healing process (B-C). In particular, DFO-treated wounds were found to reach a statistically significant higher perfusion index than vehicle-only patch and IR control wounds at post-operative day 14 (POD14) (D).**Conclusion:** Transdermal DFO demonstrates potential as a treatment modality to improve wound healing outcomes in chronically irradiated skin due to the advanced wound closure rate and elevated perfusion index noted in this study.

K4.05 | Simvastatin Cream Alleviates Dermal Fibrosis In A Rabbit Ear Hypertrophic Scar Model

David Dolivo[1], Adrian Rodrigues[1], Lauren Sun[1], Chun Hou[1], Yingxing Li[1], Eugene Chung[2], Kai P. Leung[2], Robert Galiano[1], Thomas Mustoe[1], Seok Jong Hong[1]

[1]*Surgery, Northwestern University-Feinberg School of Medicine, Chicago, IL, United States* [2]*Combat Wound Care Group, United States Army Institute of Surgical Research, San Antonio, TX, United States*

Purpose: Hypertrophic scars result from traumatic injury to the skin and represent a clinical burden with limited treatment options. Our group and others demonstrated that statin drugs (HMG-CoA reductase inhibitors) could attenuate hypertrophic scar formation in preclinical models, but convenient and topical delivery and retention of these drugs at the wound site remains a challenge.

Methods: Here we describe a cream that can deliver statin drugs and enables simple, convenient topical application, and we tested this cream in our well-characterized excisional wound model performed in rabbit ear ventral skin.

Results: We demonstrated that application of simvastatin in a cream formulation significantly attenuated hypertrophy of the resultant scars, compared to application of the identical vehicle cream without the drug. Despite causing symptoms of transient dryness and heightened trans-epidermal water loss, simvastatin cream application resulted in a decrease in macrophage and myofibroblast density at post-operative day 28 harvest. Withdrawal of statin cream treatment resulted in alleviation of dryness and substantial decrease in TEWL back to normal levels.

Conclusions: Together, these data demonstrate that cream formulation is a viable vehicle for delivery of simvastatin, which maintains its anti-fibrotic capacity, and enables rapid recovery of epidermal barrier function after its withdrawal.

K4.06 | Cerium Nitrate Treatment Diminishes Pseudomonas Aeruginosa Pathogenic Potential

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Combat Wound Care Group, United States Army Institute of Surgical Research, Converse, TX, United States

Background: Burn wound infections are significant causes of military and civilian casualties. Cerium Nitrate (CeN), a nitrate salt of cerium, a rare earth element, has been used in combination with Silver Sulfadiazine (SSD) in a cream for the management of deep burn wounds. This combination has shown added benefits over SSD alone in lessening bacterial colonization, infection, and invasion of deep burn wounds. However, details of the CeN activity on burn wound pathogens have yet to be defined. Pseudomonas aeruginosa is one of the most damaging pathogens encountered in burn wound infections. Therefore, we chose to study the impact of CeN on P. aeruginosa growth, motility, invasiveness, inflammation, and phagocytosis in vitro.

Methods: Pseudomonas growth after incubation in different concentrations of CeN at 37oC for 24 h was measured by colony-forming-units. Motility of CeN-treated Pseudomonas was measured by a swarming assay. The penetration of Pseudomonas into burned porcine skin was assessed by recovering bacteria that passed through the skin onto an underlying filter membrane (0.25 μm). In vitro inflammation and phagocytosis were studied using THP-1 monocytic cells.

Results: CeN treatment, between 0.1 to 10 mM, reduced P. aeruginosa viability dose-dependently. In the swarming assay, P. aeruginosa treated with CeN were less motile. Additionally, the penetration of Pseudomonas into the burned porcine skin was reduced by CeN treatment. In the inflammation assay, Pseudomonas treated with CeN induced lower TNF-α and IL-8 responses from THP-1 cells. Additionally, P. aeruginosa lipopolysaccharide treated with CeN was less inflammatory to ThP1 cells. Furthermore, CeN-treated P. aeruginosa (live or heat-killed) were more susceptible to phagocytosis by ThP-1 cells.

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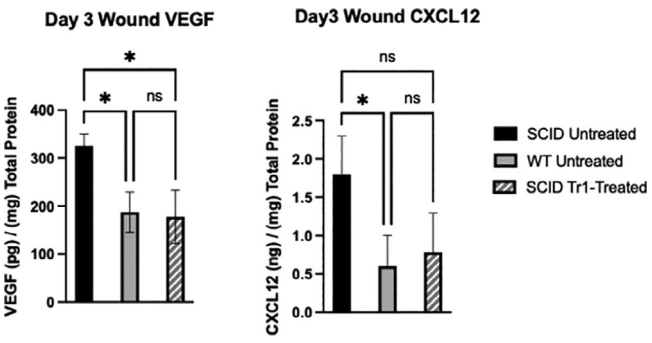
Conclusions: These results suggest that CeN treatment lessened the virulence of P. aeruginosa, making it less invasive, less pro-inflammatory, and more susceptible to phagocytosis by immune cells.

WHS SESSION N: RAPID FIRE POSTER TALKS

N1.01 | Role Of Type 1 Regulatory T-Lymphocytes In The Regulation Of Neovascularization In Dermal Wounds

Walker D. Short, Oluyinka Olutoye, Benjamin Padon, Hui Li, Ling Yu, Lily Cheng, Swathi Balaji, Sundeep G. Keswani
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Objective(s): CD4+ T-lymphocytes are essential in regulating dermal fibrosis. SCID mice, which lack lymphocytes, heal with worse angiogenesis and fibrosis compared to wild type mice. We have demonstrated that IL-10-producing type 1 regulatory T lymphocytes(Tr1) improve vascularity in the healing wound, accelerate wound closure, and reduce dermal fibrosis in SCID mice. How the immune-modulatory Tr1 regulate angiogenesis is unknown. We therefore hypothesize that Tr1 modulate the pro-angiogenic cytokine balance(VEGF; CXCL12;MIF) to improve fibroblast and endothelial cell angiogenic functions, thereby enhancing neovascularization and wound healing outcome.
Methods: 10BiT murine(M/F;8-12wk) splenocytes were sorted into Tr1 precursors and Naïve T-lymphocytes(Tnaïve). In vitro, dermal fibroblasts were co-cultured with Tnaïve or Tr1 for 24h and gene expression of VEGF and CXCL12 was measured using qRT-PCR. Dermal endothelial cells were cultured with conditioned medium from Tr1 or Tnaïve and branching assay was performed. In vivo, we made 6mm bilateral dorsal full-thickness splinted wounds on SCID or C57BL/6J(WT) mice(female,8-10wk), followed by adoptive transfer of 10^6 Tr1 cells. Wounds were harvested at days 3,7,30. D3 wound protein was measured for VEGF, CXCL12, and MIF. Angiogenic and inflammatory cellular interactions were visualized with imaging mass cytometry(IMC) at D7. D30 wounds were examined for dermal architecture and neovascularization(H&E;CD31).
Results: Fibroblasts co-cultured with Tr1 had increased expression of VEGF(1.5 fold increase;p<0.001) and CXCL12(1.5 fold increase; p<0.05) compared to untreated fibroblasts. Endothelial cells treated with Tr1conditioned medium demonstrated significantly greater branching and network formation(6.05±0.15 vs 4.37±0.56mm, p<0.05). Compared to WT, SCID mice deficient in T-lymphocytes showed abnormally elevated wound VEGF levels at D3(325.3±24.6 vs 187.4±42.1 pg/mg total protein, p<0.05), and treatment with Tr1 normalized VEGF in SCID mice to levels similar to WT(177.8±55.5 pg/mg total protein). Similarly, the positive effect of Tr1-treatment was seen in normalizing the CXCL12 expression in D3 SCID wounds(SCID:1.8 ±0.5 vs WT:0.6±0.4, SCID+Tr1:0.8±0.5 ng/mg total protein, p<0.05). MIF levels were also reduced by Tr1-treatment in D3 SCID wounds (SCID:800.6±86.2 vs SCID+Tr1 ng/mg total protein, p<0.01). IMC performed on D7 wounds revealed more mature vessels(αSMA positive) with close cellular approximation of transferred Tr1 cells, indicating their paracrine effects on angiogenesis. WT mice wounds enriched with Tr1 application to wounds at the time of wounding resulted in improved wound maturation with a basket weave collagen structure, more dermal appendages, and increased neovascularization at D30.
Conclusions: Tr1 improve the balance of angiogenic modulators VEGF, CXCL12, and MIF to promote improved neovascularization and scarring. Enrichment of Tr1 to wounds may be a therapeutic target to enhance wound healing.



N1.02 | Characterization of Mechanoresponsive Inflammatory Cells during Wound Healing

Kellen Chen, Michelle Griffin, Dominic Henn, Clark A Bonham, Katharina Fischer, Jagannath Padmanabhan, Artem A. Trotsyuk, Dharshan Sivaraj, Melissa C. Leeolou, Hudson C. Kussie, Savana L. Huskins, Sydney Steele, David Perrault, Michael T. Longaker, Geoffrey C. Gurtner
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Background: Repair after tissue injury involves a dynamic interplay among not just tissue resident cells (e.g., fibroblasts), but also cells recruited from the circulation. Myeloid cells, such as monocytes and macrophages, are derived from hematopoietic precursors and migrate to sites of injury where they play a role in modulating all stages of wound healing and scar formation. There is mounting evidence that mechanical stimuli are also able to modulate monocyte and macrophage response during tissue healing, but the exact mechanisms behind this "mechano-immunomodulation" remain incompletely understood.
Methods: We attached a mechanical strain device to the mouse dorsum to initiate a uniform and consistent strain profile across an incisional wound to create hypertrophic scar (HTS) formation in mice. To investigate mechano-responsive immune cells, we performed parabiosis of wildtype (WT) and GFP+ mice, allowed the mice to develop a shared blood circulation, initiated HTS formation in the WT mouse, and analyzed the cells using single cell RNA sequencing (scRNA-seq), fluorescent-activated cell sorting (FACS), and immunofluorescent staining.
Results: Mechanical modulation significantly upregulated the presence of inflammatory subtypes within the healing tissue, characterized

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by an increase in infiltrating GFP+ cells from 5.4% to 12.2%. In the GFP+ circulating cells, mechanical strain directly increased the proportion of fibrotic myeloid cells, primarily defined by the monocyte marker Ly6c2 as well as the TGFB responsive and macrophage activating gene Thbs1. Mechanical strain also increased the proportion of inflammatory myeloid cell populations, defined by Ccl and Il6 chemoattractants, and Cd74+ migratory myeloid cells. Utilizing both a pharmacological blocker of focal adhesion kinase (FAK) as well as a myeloid specific FAK knockout (KO), we demonstrated that modulating mechanical signaling abrogated those responses and instead promoted homeostatic myeloid transcriptional fates.

Conclusions: Tissue injury activates a cascade of signaling pathways to recruit and orchestrate various cell types during healing. Our study indicates that modulating mechanical stress directly affects myeloid cell phenotypes and interactions with other cell types in the complicated, multicellular milieu of wound healing. This principle has been previously unexplored in the context of fibrosis and regeneration, with most previous studies focused on fibroblast heterogeneity and transcriptional profiles. To our knowledge, this is the first study to directly investigate the effects of modulating mechanotransduction on immune cell response at the single cell level utilizing parabiosis and wound healing. Collectively, we demonstrate that mechano-immunomodulation of the "early responders" of healing can trigger a cascade of downstream regenerative healing.

N1.03 | Topical Treatment With Antagomir-193B-3P Accelerates Diabetic Wound Healing

Jelena Marjanovic, Ivan Jozic, Rivka C. Stone, Cheyanne Head, Beatriz Abdo Abujamra, Robert S. Kirsner, Hadar Lev-Tov, Irena Pastar, Marjana Tomic-Canic

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Background: One of the major contributing factors of healing impairment of diabetic foot ulcer (DFU) is impaired re-epithelialization marked by diminished migration and hyperproliferation of keratinocytes. We used laser capture microdissection (LCM) and genomic approaches to identify specific miRNAs that potentially can contribute to this keratinocyte non-healing phenotype and found microRNA-193b-3p (miR-193b-3p) as one of the top upregulated. Thus, we focused on deciphering the role of miR-193b-3p in epithelialization and therapeutic potential of miR-193b-3p inhibition.

Methods: We collected tissue samples from DFUs (n=15), control skin samples (n=15), and human acute wounds (n=3) and performed LCM and transcriptomic analyses using Ingenuity Pathway Analysis (IPA). qPCR was used to analyze miR-193b-3p and expression of its target genes. We performed gain- and loss-of function experiments. Cell migration was analyzed using scratch assay, and re-epithelialization was evaluated using human ex vivo and diabetic murine in vivo wound models. Formation of stress fibers involved in cell migration was studied using immunocytochemistry and RhoA pull-down assay.

Results: miR-193b-3p was found significantly induced in the epidermis of DFUs, but not in the human acute wounds. Knockdown of miR-193b-3p promoted migration of human keratinocytes, without affecting cell proliferation or cell viability. Moreover, inhibition of miR-193b-3p expression in ex vivo human wounds and diabetic murine in vivo wounds accelerated re-epithelialization. Conversely, overexpression of this miR impaired keratinocyte migration and re-epithelialization in a 3D organotypic wounds. We identified the underlying mechanism of miR-193b-3p anti-migratory activity through a disruption of stress fiber formation and a reduction of RhoA activity, with no effect on lamellipodia or filopodia formation. IPA analysis revealed miR-193b-3p target network that contributes to poor healing in DFUs. We confirmed suppression of downstream targets of miR-193b-3p in DFUs (ARHGEF6, CCND1, IGFBP5, JAK2, KIT, KRAS, MAP3K1, NFAT3, SOS2, TCF4, TNS1) and further showed that this transcriptomic landscape found in non-healing DFUs can be reversed upon miR-193b-3p suppression by topical treatment with antagomirs.

Conclusion: We identified miR-193b-3p as an important suppressor of keratinocyte migration in DFUs. Topical treatment with miR-193b-3p antagomiR represents a novel therapeutic approach for promotion of epithelialization in DFUs.

N1.04 | Aerobic Glycolysis Drives Physiological Responses To Injury Resulting In Heterogeneous Wound Repair

Oluyinka Olutoye, Fayiz Faruk, Walker D. Short, Benajmin Padon, Tanuj Prajaparti, Aditya Kaul, Hui Li, Ling Yu, Sundeep G. Keswani, Christian Coarfa, Swathi Balaji

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Background: Identical dermal injuries heal with varying degrees of fibrosis in different patients. Wound repair is an energy-intensive process, fueled by oxidative phosphorylation(OXPHOS) and glycolysis to generate ATP. Emerging evidence shows aerobic glycolysis is a preferred mechanism underlying pathologic fibrosis, but its role in physiologic wound healing is unknown. As proliferation and ECM production of fibroblasts to rebuild the dermis relies on lactate production, a prelude to shift to aerobic glycolysis, we hypothesize that differences in intrinsic fibroblast bioenergetic metabolism/aerobic glycolysis underlie differential scarring in patients.

Methods: We created a biorepository of paired normal-skin, c-section scar tissue, and fibroblasts from abdominoplasty samples from patients with clinically stratified low scar(LS) and high scar(HS) phenotypes by the Vancouver Scar Scale, <3 vs. >6 respectively. Expression of Pyruvate Kinase M2(PKM2), a modulator of aerobic glycolysis, and lactate dehydrogenase(LDHA) were measured in patient skin (IHC). OXPHOS (OCR), glycolysis (ECAR), and ATP production at resting, under stress, and with TGF-β treatment were evaluated in fibroblasts (seahorse assays), along with expression of PKM2 and LDHA (Protein

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simple WES). LS/HS human fibroblasts(106 cells/wound) were transferred to 6mm full-thickness stented wounds on SCID mice (female, 8-10weeks). Wounds were harvested on days 7 and 28. Wound closure(H&E), α-SMA, PKM2, LDHA(staining), inflammatory profile (Luminex; IHC), and collagen expression(trichrome) were analyzed. Data are presented as n=3 biologic replicates/group; p-values by ANOVA.

Results: Expression of PKM2 and LDHA was higher in both normal skin and scar tissue from HS. In vitro, RNA sequencing and GO analysis revealed several altered metabolic pathways in HS vs. LS normal skin fibroblasts. Normal skin and scar fibroblasts from HS had higher basal OCR and ECAR than LS($p<0.01$). HS fibroblasts responded to stress (FCCP/Oligomycin treatments) with significantly higher increases in spare respiratory and glycolytic reserve capacity than LS($p<0.01$), along with an increase in phospoPKM2($p<0.05$). TGF-β treatment resulted in increased phospoPKM2 and LDHA in HS vs. LS($p<0.05$). In vivo, murine wounds with HS fibroblasts showed expedited wound closure at day 7 with reduced IL-10, IL-17, MIP-1a/b, and G-CSF expression via immunoplexing and displayed increases in α-SMA (25-fold) gene expression and staining. On day 28, there were increased PKM2+ and LDHA+ spindle-shaped cells in wounds with HS, along with pronounced collagen staining and thick epidermis.

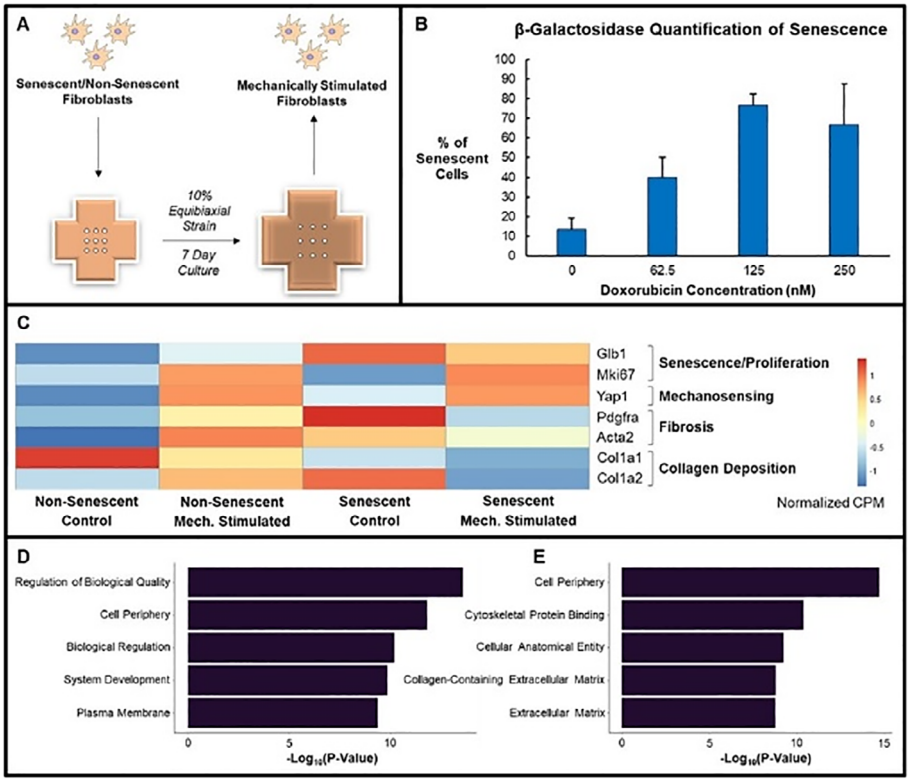
Conclusion: Fibroblasts of different scarring phenotypes display characteristic bioenergetic metabolism profiles and produce distinct scarring in murine wounds. Our data suggest that a shift to aerobic glycolysis (Warburg effect) is associated with increased fibrosis (high scar).

N1.05 | Mechanical Stimulation Reverses Pro-Fibrotic Transcriptional States in Senescent Fibroblasts

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Background: Senescent fibroblasts accumulate with age, contributing to fibrosis and impaired healing. It is of longstanding clinical interest to reverse this pro-fibrotic transcriptional state. Interestingly, senescence downregulates mechanotransduction in fibroblasts, but the effects of this phenomenon on skin fibrosis are unclear. We utilized a stretchable collagen I hydrogel (Fig. 1A) to investigate if exogenous mechanical stimulation could reverse the pro-fibrotic transcriptome of senescent fibroblasts.

Methods: Dermal fibroblasts were harvested from C57BL/6 mice by enzymatic digestion and flow cytometry, cultured in growth media, and exposed to 0-250 nM doxorubicin (72h) to induce senescence, quantified by β-galactosidase. Fibroblasts were encapsulated (1,000,000 cells/mL) in 1.92 mg/mL collagen I, 0.8x MEM, and 16mM HEPES. Gels were formed in 20x20x5mm cruciforms and cultured 7 days (media replaced each 2-3 days) with 10% equibiaxial strain for mechanical stimulation. RNA was retrieved by collagenase digestion and TRIzol extraction. RNAseq was performed (40M paired-end reads), aligned to mm10 (HISAT2), and quantified (StringTie). Differential expression analysis and gene ontology (GO) analysis of the top 500 differential genes were performed (edgeR; α=0.05). All experiments performed at n=3.



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Results: β-galactosidase showed optimal 76.7% senescence induction with 125nM doxorubicin (Fig. 1B). RNAseq showed increased Glb1 (senescence) and decreased Mki67 (non-senescence/proliferation) expression for senescent fibroblasts, which were reversed by mechanical stimulation (Fig. 1C). Yap1 (mechanosensing) expression increased with mechanical stimulation of non-senescent and senescent fibroblasts. Pdgfra and Acta2 (pro-fibrotic, canonical) were highly enriched in senescent fibroblasts. Critically, these pro-fibrotic markers were reversed by mechanical stimulation in senescent fibroblasts only. Col1a1 was downregulated by senescence and mechanical stimulation, while Col1a2 was downregulated by mechanical stimulation of senescent fibroblasts only.

GO analysis revealed that mechanical stimulation differentially modified transcriptomes of non-senescent and senescent fibroblasts. The top 5 enriched terms for senescent fibroblasts were characterized by systemic modifications to biological quality/development (Fig. 1D), while the top 5 enriched terms for non-senescent fibroblasts were largely superficial responses to stretched matrix/gel (Fig. 1E).

Conclusions: Mechanical stimulation differentially modified normal vs. senescent fibroblasts, increasing pro-fibrotic transcription for non-senescent fibroblasts but reversing pro-fibrotic transcription and systemically modifying biological qualities for senescent fibroblasts. Furthermore, mechanical stimulation mitigates inherent features of the senescent phenotype (Glb1+/Mki67-). Ultimately, our model suggests that exogenous mechanical stimulation reverses the pro-fibrotic phenotype of senescent dermal fibroblasts.

N1.06 | Endothelial PLCG2: The missing link that makes VEGF Therapy Robust

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ICRME, Department of Surgery, Indiana University, Indianapolis, IN, United States

Critical limb threating ischemia (CLTI) is a severe form of peripheral arterial disease with markedly reduced blood flow in lower extremities. Type 2 diabetes mellitus increases the incidence and severity of CLTI by 2-4-fold. Hyperglycemia induced endothelial cell dysfunction is recognized as a contributor to such limitation. For CLTI, VEGF therapy has met with limited success. To address such limitation, we applied single-cell RNA sequencing (scRNA-seq) technology to study the endothelial cells of the human diabetic skin. Single-cell suspensions were generated from the human skin followed by cDNA preparation using Chromium Next GEM Single Cell 3' Gel Bead Kit v3.1(10x Genomics, Inc.) and sequenced on NovaSeq 6000 (Illumina, Inc). Using our published quality control measures, 36,487 cells (from 3 diabetic skin and 5 non-diabetic skin) were chosen for downstream analysis. Global communications analyses of scRNA-seq data using CellChat identified that although VEGF signaling was not significantly altered in diabetic vs non-diabetic skin, phospholipase C Gamma 2 (PLCG2) was significantly down-regulated. The significance of PLCG2 in VEGF mediated increase in endothelial mitochondria function was assessed in cultured human microvascular endothelial (HMEC) cells using extracellular flux (Seahorse™) assay. In HMEC, VEGF enhanced mitochondrial function as indicated by elevation in oxygen consumption rate (OCR) and extracellular acidification rate (ECAR). PLCG2 inhibition impaired the action of VEGF on mitochondrial metabolism ($n = 5$, $^*P < 0.05$, one-way ANOVA). Follow-up rescue studies therefore focused on understanding the significance of VEGF therapy in presence or absence of endothelial PLCG2 in murine diabetic ischemic tissue. Non-viral tissue nanotransfection technology (TNT2.0) mediated delivery of PLCG2 to endothelial cells was achieved through cadherin-5 promoter driven PLCG2-ORF. Targeted delivery of endothelial PLCG2 promoted the rescue of hind-limb ischemia in diabetic mice ($n = 6$, $^*P < 0.05$, one-way ANOVA). Improvement of blood flow was also associated with higher abundance of VWF+/CD31+ and VWF+/SMA+ immunohistochemical staining ($n = 6$, $^*P < 0.05$, one-way ANOVA). TNT-based gene delivery was tolerated well by diabetic ischemic tissue. Taken together, this work identifies endothelial PLCG2 as an important contributor to the success of VEGF therapy in a diabetic ischemic limb setting.

N1.07 | Microfibril Associated Protein 5 in Skin Wound Healing

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Recent studies from our lab demonstrated that fibroblasts are phagocytic in healing skin wounds. RNA sequencing of phagocytic fibroblasts led to the identification of Microfibril associated protein 5 (MFAP5, or microfibril-associated glycoprotein 2/MAGP2) as a molecule that is significantly unregulated in phagocytic fibroblasts. MFAP5, a 25 kD serine and threonine rich small protein, is a component of microfibrils in the extracellular matrix (ECM). MFAP5 is known to be closely associated with fibrosis and angiogenesis in certain cancers. Given the role of MFAP5 in cancer, the aim of this study was to investigate the production of MFAP5 in fibroblasts and in a murine model of skin wound repair. Flow cytometric analysis demonstrated that skin fibroblasts constitutively express MFAP5 and that MFAP5 levels increase markedly in actively phagocytic fibroblasts. Microarray and real time PCR analysis of full thickness excisional wounds showed that MFAP5 mRNA expression is significantly upregulated during the proliferative and remodeling phases of healing (day 3 to 21). Immunohistochemical staining demonstrated that MFAP5 was strongly present in the cytoplasm of dermal cells and in the dermal ECM of scars. In addition, MFAP5 was found to be strongly expressed in human keloids. The data shows that fibroblasts can be a source of MFAP5 in

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wounds, and that the expression of MFAP5 changes significantly over the course of skin wound healing. The findings support a role for MFAP5 in the regulation of fibroblasts, scar formation, and ECM function in skin wound healing.

Supported by NIH R01 GM50875, R35 GM139603-01, and F31 DE028747-01

N1.08 | Cellular vs. Acellular Tissue-Based Products For The Treatment Of Diabetic Foot Ulcers: Final Report Of A Longitudinal Comparative Effectiveness Study

Harrison J. Shawa[1], Ramajot Kaur[2], Catherine Tchanque-Fossuo[2], Hadar Lev-Tov[2], Kaitlyn West[2], Nuen Yang[3], David Rocke[3], Sara Dahle[4], Rivkah Isseroff[1]

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Diabetic foot ulcers (DFU) present a significant challenge to the United States healthcare system. With the current of standard of care (SOC), less than 30% of wounds heal after 20 weeks. This dismal cure rate prompted development of advanced therapies, albeit with great cost and only modest improvement, with limited data showing even 50% of wounds healing within 12 weeks. While the cost of these newly developed approaches varies widely, studies comparing them directly against each other have not been done. We addressed this knowledge-gap via comparative analysis of effectiveness between a cellular matrix (Dermagraft®) and an acellular matrix (Oasis®), hypothesizing no difference in healing effected by either matrix.

This randomized, single-blinded, controlled clinical trial compared DFU-healing effectiveness of two intervention arms: cellular matrix and acellular matrix, and SOC. Over 600 patients were screened, yielding 120 randomized patients from October 2011 – February 2017. Subjects in either intervention arm received up to 8 weekly matrix applications. 103 patients reached the primary endpoint of 12 weeks. 96 patients reached the secondary endpoint of 31 weeks. No significant inter-group demographic or wound characteristic differences were noted (p-values>0.05). 57.14% and 73.53% in the cellular matrix group, and 78.57% and 69.23% in the acellular matrix group healed by 12 and 31 weeks, respectively (p-values >0.05). In the SOC group, 61.2% and 78.3% of wounds healed by 12 and 31 weeks, respectively. The average rate of ulcer healing among three treatment arms was similar (p>0.05). The average wound size in each treatment arm at week 1, week 15, and week 19 were similar (p>0.05). No association was found among offloading methods among groups (p=0.87). The cost of the cellular and acellular matrices was $1081.50 and $107.57 per application, respectively.

This comparative analysis reveals that treatment effected by cellular and acellular matrices is comparable at both 12 and 31 weeks, suggesting that the acellular matrix is a more cost-effective treatment than the cellular matrix.

WHS SESSION O: CONCURRENT ORAL ABSTRACTS III (non-accredited)

O1: Directed Energy Therapies

O1.01 | Wireless Electrotherapy Improves Healing Of Infected Wounds In Porcine Model

Maksym Krutko[1], Nava Rijal[1], Rebecca Ruppel[1], Taylor Wallen[3], Michael Goodman[3], Andrei Kogan[2], Melanie Cushion[4], Daria Narmoneva[1]

[1]Biomedical Engineering, University of Cincinnati, Cincinnati, OH, United States [2]Physics, University of Cincinnati, Cincinnati, OH, United States [3]Surgery, University of Cincinnati, Cincinnati, OH, United States [4]Internal Medicine, University of Cincinnati, Cincinnati, OH, United States

Background: Poor vascular supply and infection are significant factors in impaired healing of chronic wounds. We have developed a non-contact wireless electric field (EF) therapy that can stimulate cells within the entire wound. An optimal therapeutic signal for the in vivo experiments is determined using numerical in-situ simulation (ANSOFT/HFSS) for the interactions between the applied EF and underlying wound tissues. The objective of this study is to determine efficacy of this wireless electrotherapy on healing of infected wounds in the porcine model.

Hypothesis: the wireless electrotherapy enhances vascularization, reduces bacterial load and promotes wound microenvironment that is conducive to healing.

Methods: N=14 wounds (2x2 cm) were created on dorsum of two female white Yorkshire pigs. The wounds were divided into four groups: electrotherapy vs. untreated control, each with or without infection (n=3-4 wounds per group). Staphylococcus aureus (SA) and Pseudomonas aeruginosa (PA) were delivered post-wounding (1x10e9 of each bacteria) in 0.3mL total volume per wound. Control wounds were inoculated with sterile PBS only. Wounds were covered with TegadermTM dressing. Electrotherapy was applied 1 hr/day, 3-5 days/week, for 4 weeks. The effects of the electrotherapy on wound closure, bacterial load (scab or swab analyses), and gene expression (RNAseq analyses) were quantified.

Results: Electrotherapy resulted in significant reduction in the wound area at 2 and 3 weeks post-surgery for both infected and control (non-infected) wounds (p<0.001) and significantly faster wound closure (p<0.01). The results demonstrate significantly reduced levels of both types of bacteria in electrotherapy-treated wounds at 2 weeks post inoculation. Genomic analysis revealed significant upregulation in key pathways and individual genes responsible for wound healing such as angiogenesis, antibacterial humoral response, ECM remodeling, and activating immune responses (p<10-4-10-6, ssGSEA and DEseq), with analyses currently ongoing. Successful application of the

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combined experimental approach demonstrates that wireless electrotherapy enhances infected wound healing via deep stimulation of wound tissues, increasing rate of wound closure, reducing bacterial colony count, and upregulating genes wound healing related genes.

Conclusions: The results demonstrate that wireless electrotherapy is effective in reducing wound infection and improving healing, suggesting the promise of this strategy to improve healing outcomes and decrease amputations in patients with infected non-healing ulcers.

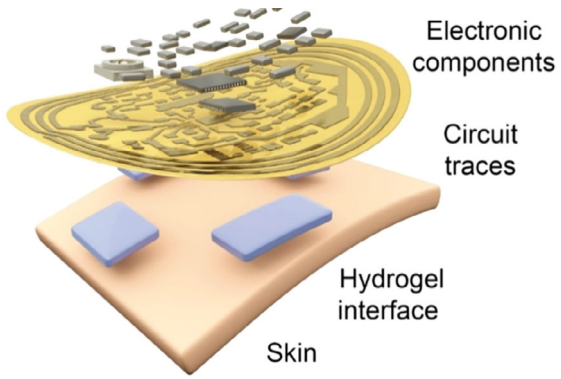
O1.02 | Galvanotactic Smart Bandage for Chronic Wound Management and Tissue Regeneration

Artem A. Trotsyuk, Yuanwen Jiang, Simiao Niu, Dominic Henn, Kellen Chen, Maddy Larson, Ethan Beard, Aref Saberi, Dharshan Sivaraj, Alana Malaika Mermin-Bunnell, Smiti Mittal, Serena Jing, Sun Hyung Kwon, Clark Bonham, Jagannath Padmanabhan, David Perrault, Melissa C. Leeolou, Michael Januszyk, Zhenan Bao, Geoffrey C. Gurtner

Surgery, Stanford University, Stanford, CA, United States

Background: Approximately thirty million people in the United States suffer from diabetes. The prevalence of diabetic foot ulcers (DFUs) in this population is 13%. Current standard of care wound dressings are passive and cannot actively respond to variations in the wound environment. Smart bandages are well positioned to address these challenges. To our knowledge there have not been significant advancements in incorporating sensing technologies to deliver active wound care. We believe this can be achieved using a multidisciplinary approach combining electrical and chemical engineering with the fundamentals of cellular and biomolecular processes in wound healing directed towards high resolution, in situ tissue regeneration.

Methods: A flexible printed wireless stimulator was designed and fabricated to deliver directional energy across a wound gradient. Subsequently a low impedance PEDOT:PSS electrode was designed to optimize the skin and stimulator interface, producing a robust gel with tunable adhesion properties. The smart bandage was evaluated in an excisional diabetic and C57BL6/J murine wound healing model. A parabiosis model was used to evaluate circulating cell migration into the wound bed. Single cell analyses were performed to evaluate changes in cell populations as a direct result of induced electrical stimulation. In vitro validation was performed to elucidate in vivo results.



Results: Wireless electrical stimulation resulted in significantly accelerated wound closure, when compared to controls, in both a diabetic and C57BL6/J murine excisional wound healing model. Complete epidermal recovery was observed, with a thicker collagen network and increased dermal thickness. Greater neovascularization and appendage formation were observed in the treatment groups. Single cell analyses revealed higher proliferation and remodeling regulatory markers expressed across treated groups. In vitro co-culture validation experiments demonstrated accelerated proliferation, mitotic rate and tube formation when compared to controls.

Conclusion: Our data demonstrates the functionality of a robust wireless interface for wound healing. This novel treatment modality will integrate AI processing components for the development of a closed-loop functional stimulator. By combining the domain expertise of nanofabrication, mechanotransduction, fibrosis and molecular/cellular analyses, we are developing a novel chronically stable and robust smart bandage that will pave the way for the next generation of palliative wound care.

O1.03 | Using Membrane Acting Agents And Ultrasound To Overcome Biological Barriers And Eradicate A Methicillin Resistant Staphylococcus Aureus Wound Infection In Diabetic Mice

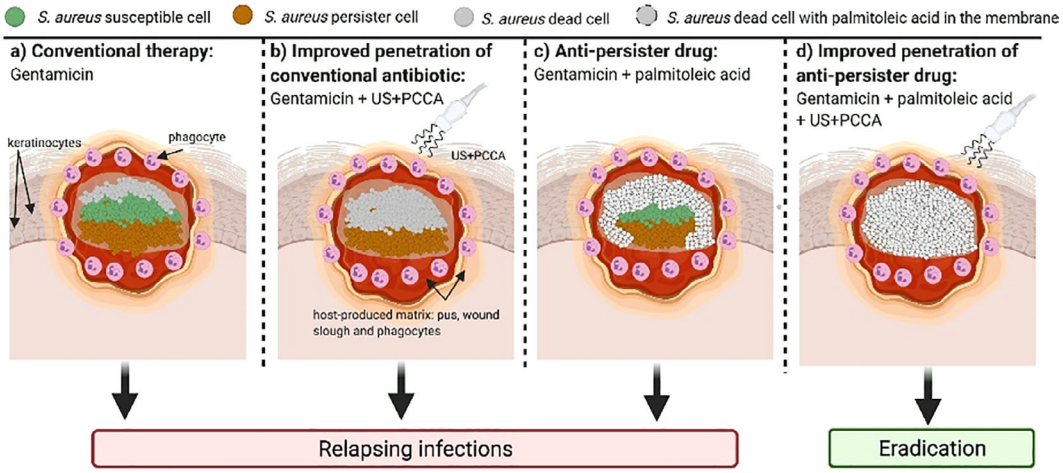
Sarah E. Rowe-Conlon[1], Ashelyn Sidders[1], Phillip Durham[2], Paul Dayton[2], Brian Conlon[1], Virginie Papadopoulou[2]

[1]Microbiology and Immunolgy, UNC Chapel Hill, Chapel Hill, NC, United States [2]Joint Department of Biomedical Engineering, University of North Carolina and North Carolina State University, Chapel Hill, NC, United States

Bacterial biofilms are the leading cause of delayed healing in chronic wounds. Biofilms display remarkable tolerance to antibiotics as they impede penetration of drugs and even when drugs do penetrate, cells within a biofilm are in an antibiotic tolerant state. Aminoglycosides, such as gentamicin (Gent), are ineffective against biofilm cells as they maintain proton motive force below the threshold for drug uptake. We employ a novel aminoglycoside adjuvant, palmitoleic acid (PA), to facilitate drug uptake. Here we propose a dual strategy to eradicate a chronic wound infection; utilizing ultrasound-stimulated phase change contrast agents (US+PCCA) to improve the penetration of the novel drug combination Gent/PA.

A chronic wound infection model was established in SKH-1 hairless mice; 6-8 week old mice were treated with streptozocin to induce diabetes. A circular wound was created on the back of the mice and infected with methicillin resistant Staphylococcus aureus (MRSA). Wounds were treated topically with Gent and/or PA, twice daily for 4 days. One daily antibiotic treatment was combined with US+PCCA. On day 5, mice were euthanized, the wound area was excised and plated to enumerate bacterial survivors.

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Neither Gent nor Gent/PA reduced bacterial burden but both treatments were significantly improved by applying US+PCCA to improve drug penetration (Kruskal Wallis One-Way ANOVA with Dunn's multiple comparison test). Importantly, bacteria were eradiated from 3 out of the 8 wounds in the Gent/PA group that were treated with US+PCCA.This data shows that improving penetration of a novel anti-biofilm drug combination is a viable strategy to eradicate biofilms in chronic wounds. Palmitoleic acid is an attractive antibiotic adjuvant as it has previously shown to reduce inflammation and accelerate wound healing. This therapeutic strategy of utilizing ultrasound to improve penetration of anti-biofilm drugs has the potential for clinical translation as ultrasound is already used in wound debridement and the PCCA formulation is a variant of FDA approved contrast agents currently used in diagnostic imaging.

O1.04 | Fibroblast Clearance Of Damaged Tissue Following Laser Ablation Of Engineered Microtissues In Vitro

Megan Griebel, Anish Srinivas Vasan, Christopher Chen, Jeroen Eyckmans
Boston University, Brookline, MA, United States

Background: Clearance of damaged and necrotic tissue is a critical step for wounds to heal. Although the role of immune cells, such as neutrophils and macrophages, in the autolytic debridement of wounds is appreciated, it is unclear if fibroblasts are able to contribute to this process. Herein, we demonstrate fibroblast-mediated wound clearance following laser ablation of engineered microtissues prior to wound closure.

Methods: Collagen type I laden with 3T3 fibroblasts is added to polydimethylsiloxane (PDMS) micro-wells, each containing four PDMS pillars. Following collagen compaction, a rectangular (1 mm X 0.6 mm) microtissue is suspended between the pillars. Full-thickness wounds between 100 mm and 200 mm in diameter are generated either mechanically, using a microdissection knife, or by ablation, using a nanosecond-pulsed Nd:YAG laser. Following injury, live microscopy is used to track and quantify the change in wound area. To study the role of cytoskeletal dynamics in clearance of the wound extracellular matrix (ECM), inhibitors of cellular contractility (Y-27632, blebbistatin, MLCK inhibitor peptide-18), microtubule polymerization (Nocodazole), and clathrin-mediated endocytosis (dynasore) were added to the microtissue prior to injury.

Results: Ablated tissues displayed significantly more cell death (up to 10% of tissue area) at the wound perimeter than microtissues injured using a microdissection knife (<1% by area). Microtissues injured by knife displayed smooth wound edges and began closure (marked by a decreasing wound area) within 2 hours following injury. In contrast, ablated microtissues first displayed wound opening for an average of 6 hours before closure commenced. The rate of tissue opening was decreased by 28% ($p<0.001$) and 26% ($p<0.001$) when microtissues were treated with Y-27632 and dynasore, respectively. Transmission electron microscopy imaging of injured microtissues showed elevated number of vesicles containing collagen fibrils, thus suggesting a role for endocytic processes during wound clearance by fibroblasts.

Conclusions: By using laser ablation, we are able to generate wounds in vitro that contain damaged tissue, as might be seen in burn wounds or chronic wounds where debridement is impeded. Our data suggest that fibroblasts are capable of clearing damaged tissue prior to wound closure, a process that is mediated by ROCK activity and endocytosis.

O1.05 | Sustained Electroceutical Therapy Of Chronic Ischemic Wounds Decreases Infection And Accelerates Healing

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[2] *Case Western Reserve University, Cleveland, OH, United States*

Background: Patients with chronic wounds often need to make multiple trips to the clinic for specialised wound care. The annual cost to treat these wounds has been estimated by the Agency for Healthcare Research and Quality to be $9.1–$11.6 billion per year. Electrotherapy is well established as a biophysical treatment modality for chronic wounds but is not currently reimbursed. Following rigorous evaluation of the wide variety of clinically reported electroceutical parameters,

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we developed exciflex, a disposable occlusive bandage together with a reusable stimulation/control module. The absorbent, flexible, and transparent substrate material provides visualization of the wound bed without needing to remove the bandage with management of wound exudate, identified as a need by our clinical collaborators.

The primary outcomes of interest were that wounds treated by exciflex would exhibit improved healing and decreased infection.

Methods: The exciflex bandage was evaluated in a pre-clinical porcine model of large chronically infected wounds, with electroceutical therapy applied at a 10% duty cycle, i.e. for 6 minutes every hour. Control wounds were treated with standard of care dressings. Bandage changes for all wounds and wound monitoring was carried out at timepoints relevant to the wound healing pathway until the wounds were healed. Bandage changes and wound monitoring was carried out at timepoints relevant to the wound healing pathway until the wounds were healed. Wound status monitoring included digital stereophotogrammetry together with evaluation of planktonic bacterial infection and biofilm sampling

Results: The reusable exciflex stimulation/control module was robust and remained fully functional in the challenging environment of the porcine model. Wounds treated by exciflex healed by 80% after 10 days of treatment, a 20% improvement over standard of care. Wound infection and biofilm were significantly decreased in wounds treated by exciflex ($p<0.001$). 16S rRNA sequencing analysis indicated that biofilm diversity also decreased over time in these wounds.

Conclusions: The exciflex bandage can be worn for up to one week so that therapy can be delivered around the clock and is not limited to clinic visits, which promotes healing and increases patient safety. Prior work provides the base for an upcoming first-in-man trial. The BRAVE study will provide an essential translational step for exciflex.

O1.06 | Electroceutical Technology Against Wound Microbial Biofilm Infection

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Background: Wound biofilms are a challenging and perennial problem encountered by physicians. The CDC estimates that 65% of all human infectious disease is caused by bacteria with a biofilm phenotype and NIH estimates that this number is closer to 80%. Surgical approaches are temporarily productive, however, remain ineffective in eradication of this problem. Electrical principles influence fundamental processes in bacterial biofilm formation biology. In past few years, we have reported the efficacy of electroceutical-based wound dressing (EDTlo and EDThi) treatment in vitro biofilm infection and in vivo wound infection models.

Objective: The objective of this work is to develop and optimize an electroceutical dressing based protocol for management of biofilm infected clinical chronic wounds.

Methods: An established pre-clinical porcine burn wound biofilm model infected with Pseudomonas aeruginosa (PA01) and Staphylococcus aureus USA 300 (MRSA) was used to establish chronic biofilm infection in wounds. Full-thickness burn wounds (2"x2") infected with biofilm were treated with EDThi for 28 days post-infection followed by EDTlo until the end of the study (d56). The EDT dressings were changed twice a week throughout the duration of the study (d56). Placebo group included treatment with EDhi (power off) and EDlo (silver only) dressings. Analyses of wound closure (digital planimetry), skin barrier function (TEWL), wound biofilm infection assay (by scanning electron microscopy, immunohistochemistry) were performed.

Results: Scanning electron microscope (SEM) images of d14 post-infected wound biopsies showed thick aggregates of bacteria encased in extrapolymeric matrix (EPS) characteristic of bacterial biofilm. The biofilm structures significantly reduced in the EDT treated wounds. Confocal fluorescence microscopy analysis of biofilm infection in wounds exhibited diminished bacterial aggregates in the EDT treated wounds indicative of reduction in biofilm infection. Next, the effect of EDT treatment on host responses was investigated. A significant ($p<0.05$; n=6) improvement in wound closure accompanied by a robust ($p<0.05$; n=6) reepithelialization (keratin 14 stating) response was noted in EDT treatment group as compared to sham treated group suggesting a clear improvement in host wound repair response.

Conclusion: The data indicated that electroceutical treatment protocol effectively eradicated wound biofilm infection and improved wound repair response in host suggesting therapeutic use of EDT dressings in recalcitrant biofilm infection.

O2: Wound Physiology

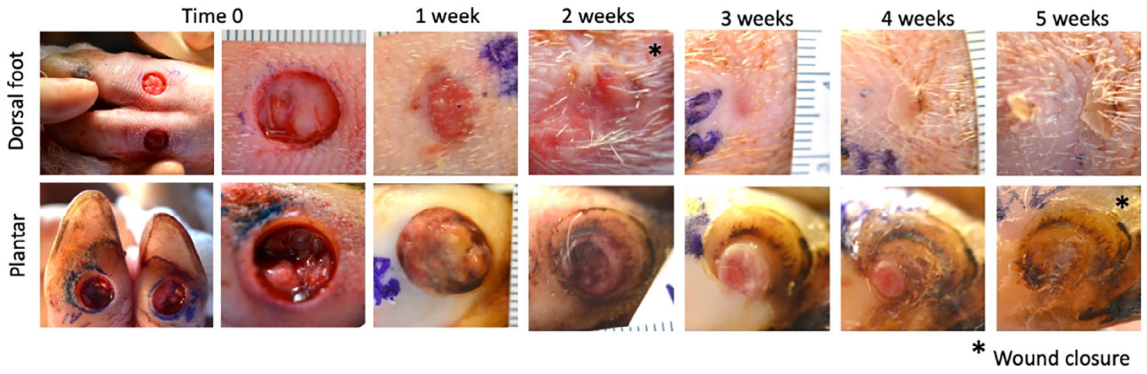
O2.01 | Unique Physiology And Intrinsically Delayed Wound Healing In Plantar Skin

Christiane Fuchs[1], Katherine J. Stalnaker[1], Linh Pham[1], Ying Wang[1], Clifton L. Dalgard[2], Sunghun Cho[3], Jon H. Meyerle[3], Joshua Tam[1]
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Background: Diabetic foot ulcers (DFUs) have devastating health and socioeconomic impacts. Previous research in DFU has focused primarily on diabetes-related pathologies, while the "foot" aspect has been mostly neglected, despite the many well-characterized specializations of plantar skin, including lack of hair, unique epidermal structural, accelerated epidermal turnover, poor barrier function, and keratin 9 expression. In this study we investigated how plantar-specific properties could impact wound healing.

Methods: This study utilized the porcine model because of anatomic and physiologic similarities between porcine and human skin. Skin samples were collected from the trunk, snout, plantar and dorsal foot. Structural features were characterized by standard histology and

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transmission electron microscopy. Tissue calcium distribution and calcium signaling in primary keratinocytes were evaluated using calcium indicator dyes. Gene expression profiles were compared by RNAseq. Immunohistochemistry confirmed protein expression and distribution in the porcine skin samples, with key findings validated in human plantar and abdominal skin samples. A new porcine plantar wound model was developed to compare the wound healing process between plantar, trunk, and dorsal foot skin.

Results: Plantar skin showed many unique characteristics, including greatly thickened epidermis composed of hypertrophic keratinocytes, a compact stratum corneum that is structurally distinct from non-plantar skin, absence of epidermal calcium gradient (thought to be critical for keratinocyte differentiation), and a gene expression profile that clusters away from other skin types, but shares certain similarities with activated wound keratinocytes. Of particular interest was stromal interaction molecule 1 (STIM1), which showed diminished protein expression in both porcine and human plantar skin. STIM1 is a key regulator of intracellular calcium storage, and a critical component of the store-operated calcium entry (SOCE) response which, due to its ubiquitous utility in calcium second messenger signaling, is involved in many wound healing processes. Reduced STIM1 in plantar epidermis is consistent with its diminished calcium gradient, and predicts the blunting of SOCE and associated downstream pathways. Using primary keratinocytes, we verified that plantar keratinocytes have a substantially diminished SOCE response. Finally, wound contraction and re-epithelialization are both delayed in porcine plantar wounds, causing wound closure time to more than double that of non-plantar control wounds.

Conclusions: We have found that plantar skin is more distinctive from "normal" skin than previously realized, with intrinsically delayed wound healing, and that these properties are likely related to altered calcium signaling. The atypical physiology and wound healing process in plantar skin are likely to contribute significantly to the pathology of plantar ulcers.

O2.02 | Genomic And Phenotypic Fibrotic Cutaneous Response To Radiation Injury Extends Beyond The Treatment Field In A Murine Model Of Radiation-Induced Skin Fibrosis

Nathan Balukoff[1], Jamie L. Burgess[1], Tongyu Wikramanayake[1], Gregory Azzam[2], Stuart Samuels[2], Sharon Elliot[3], Marjana Tomic-Canic[1], Rivka C. Stone[1]

[1]Dr. Phillip Frost Department of Dermatology and Cutaneous Surgery, University of Miami Miller School of Medicine, Miami, FL, United States [2]Department of Radiation Oncology, University of Miami Miller School of Medicine, Miami, FL, United States [3]Department of Surgery, University of Miami Miller School of Medicine, Miami, FL, United States

Progressive skin fibrosis develops in a subset of patients following high-dose radiation exposure in the oncology and interventional radiology settings. Radiation-induced skin fibrosis is a debilitating disease with devastating progression that results in long-term disability, discomfort, and negative impact of quality of life. For this study we sought to establish a murine model in which fibrosis is induced by high-dose irradiation of skin. C57BL/6 wild-type mice (n=6) were irradiated with a single dose of 18 Gy for 8 minutes, maintained under standard conditions for 24 weeks, and sacrificed for collection of skin from irradiated and adjacent non-irradiated skin and compared with non- irradiated murine controls (n=3). To quantify fibrosis, dermal thickness was measured in hematoxylin and eosin (H&E)- and Masson Trichrome-stained tissue sections. Hydroxyproline content was measured via colorimetric assay (Sigma) as an assessment of total collagen levels. RNA-Sequencing of murine skin from irradiated and non-irradiated sites was performed and compared with untreated controls, and transcriptomic profiles were analyzed using Partek Flow (Partek Inc.) and Ingenuity Pathway Analysis (Qiagen) for pathway enrichment analyses. We observed that in addition to developing alopecia and hypopigmentation at the irradiated site, mice developed histologic evidence of fibrosis as demonstrated by increased dermal thickness on H&E and Masson Trichrome-stained sections ($p<0.005$). There was no significant difference in hydroxyproline (collagen) content in irradiated versus non- irradiated areas of each mouse; however, collagen was increased in irradiated mice compared with untreated (non-irradiated) controls ($p<0.05$). Expression of 665 fibrosis-associated genes were similar in RNA-seq profiles from irradiated and non-irradiated areas, but distinct from mice that had no exposure to radiation. In conclusion, we successfully induced fibrosis in murine skin via delivery of high-dose radiation as the source of injury. Fibrosis in irradiated mice was evidenced by increased dermal thickness, increased hydroxyproline content, and evidence of fibrosis-associated gene and pathway induction at the genomic level in RNA-seq profiles. Notably, the irradiated and non-irradiated areas of each mouse appeared phenotypically distinct, but the pro-fibrotic effects of radiation extended beyond the

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treatment field. Evidence of fibrosis in areas outside of the radiation treatment field highlight the importance of prophylactic and therapeutic approaches to minimize off-target effects and to prevent severe progressive skin fibrosis following radiotherapy.

O2.03 | Cellular Mechanism of Highly-Active or Antiretroviral Therapy-Induced Lipodystrophy: Atazanavir, A Protease Inhibitor, Compromises Adipogenic Conversion of Adipose-Derived Stem/Progenitor Cells through Accelerating ER Stress-Mediated Cell Death in Differentiating Adipocytes

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Fukushima Medical University/Jonaikai, Shimabara, Nagasaki, Japan

Background: Lipodystrophy is a common complication in human immunodeficiency virus (HIV)-infected patients receiving highly active antiretroviral therapy (HAART) or antiretroviral therapy (ART). Previous studies demonstrated that endoplasmic reticulum (ER) stress-mediated unfolded protein response (UPR) is involved in lipodystrophy; however, the detailed mechanism has not been fully described in human adipogenic cell lineage.

Methods: Adipose tissue-derived stem cells (ADSCs) obtained from human subcutaneous adipose tissue was used, and atazanavir (ATV), a protease inhibitor (PI), was administered to ADSCs and ADSCs undergoing adipogenic conversion.

Results: Greater repression of adipogenic differentiation was observed when ATV was administered during 10 days of ADSC culture in adipogenic differentiation medium. Although ATV had no effect on ADSCs, it significantly induced apoptosis in differentiating adipocytes. ATV treatment also caused the punctate appearance of CCAAT-enhancer-binding (C/EBP) protein homologous protein (CHOP), and altered expression of CHOP and GRP78/Bip, which are the representation of ER stress, only in differentiating adipocytes. Administration of UPR inhibitors restored adipogenic differentiation, indicating that ER stress mediated UPR was induced in differentiating adipocytes in the presence of ATV. It is also observed autophagy, which was potentiated in differentiating adipocytes by ATV treatment. Thus, adipogenic cell atrophy leads to ATV-induced lipodystrophy, which is mediated by ER stress-mediated UPR and accelerated autophagy, both of which would cause adipogenic apoptosis.

Conclusion: As our study demonstrated for the first time that ADSCs are unsusceptible to ATV and its deleterious effects are limited to the differentiating adipocytes, responsible target(s) for ATV-induced lipodystrophy may be protease(s) processing adipogenesis-specific protein(s).

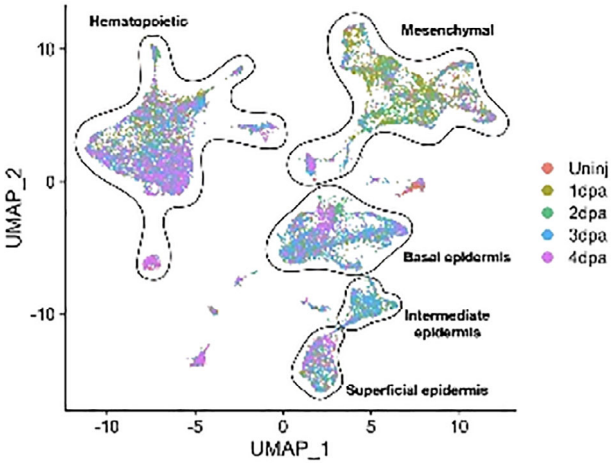
O2.04 | The Role Of Sp Transcription Factors In Appendage Regeneration, From Fins To Feet

David A. Brown[1], Grant Darner[2], Timothy Curtis[2], Jianhong Ou[2], Thomas Dvergsten[2], Veronica Han[2], Sydney Jeffs[3], Kenneth Poss[2]

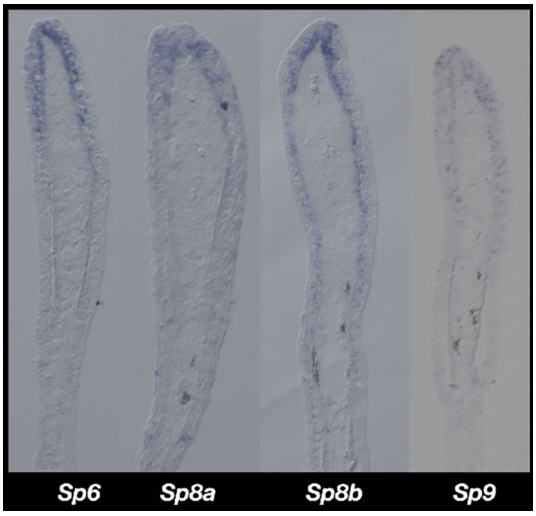
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Background: A first critical step in limb or appendage regeneration is re-epithelialization of the amputation stump to form a specialized regeneration epidermis, which functions as a signaling center to initiate reformation of the structure from underlying mesenchyme. We hypothesized that common factors exist in the regeneration epidermis among species with innate abilities in appendage regeneration.

Methods: The regeneration epidermis of zebrafish caudal fin was profiled with single-cell RNA sequencing. Immunofluorescence and in situ hybridization were used to characterize gene expression. Zebrafish null mutants were generated with the CRISPR/Cas-9 system. Epidermal-specific, inducible deletion mutants in mice were produced by

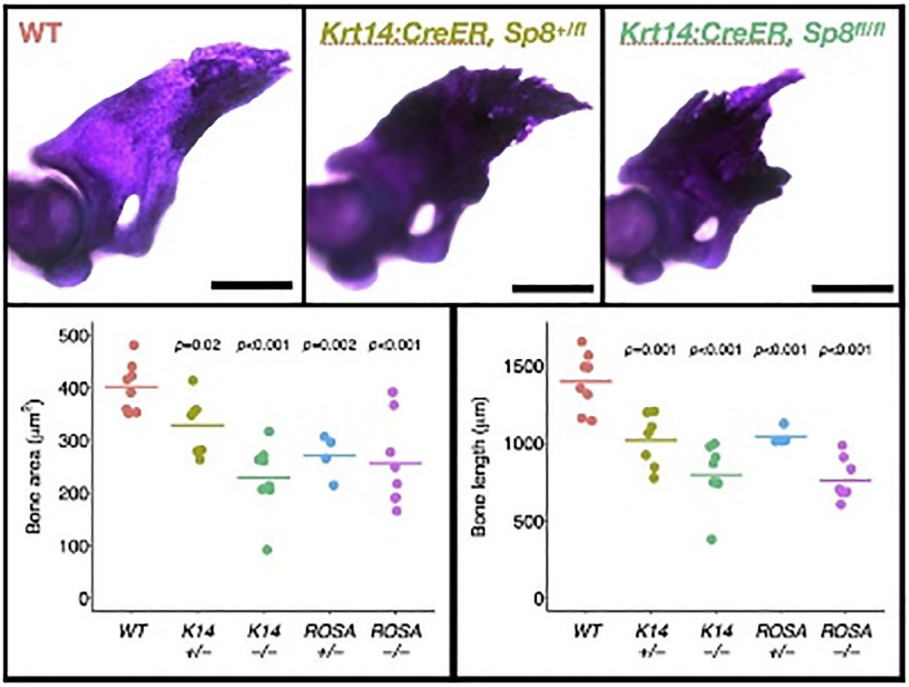


UMAP clustering of single-cell RNA expression in regenerating zebrafish caudal fin



In situ hybridization demonstrates localization of Sp family expression to the epidermis in regenerating zebrafishcaudal fin, 4 days post amputation

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Epidermal-specific, inducible deletion of Sp8 produces incomplete digit regeneration in mice.

crossing mice expressing CreER under the Krt14 promoter to mice harboring floxed alleles of the target genes.

Results: Single-cell RNA sequencing in regenerating zebrafish fin revealed an enriched cell cluster marked by the transcription factor Sp6. Immunofluorescence and in situ hybridization confirmed the expression of Sp6 and other Sp family members in the basal epidermis of regenerating caudal fin as well as regenerating mouse digits. CRISPR/Cas-9 was used to generate null mutants in zebrafish for several Sp genes, which demonstrate a variety of skeletal defects and lethality. Mice deficient for Sp6 and Sp8 exhibit distal limb defects, while induced deletion of Sp8 in the basal epidermis of adult mice before digit amputation display impaired bone outgrowth, suggesting a critical role in this process. Intriguingly, we also detected Sp6 and Sp8 in the wound epidermis of human leg amputations. Upcoming work will identify transcriptional targets of Sp8 in regenerating mouse digits and human leg amputation wounds with CUT&RUN sequencing.**Conclusions:** Sp transcription factors may represent conserved genes that participate in appendage regeneration across various species. Future work will define the role of these factors in regeneration-linked gene expression, as well as explore the idea that their expression may be remnants of an incomplete limb regeneration program in humans.

O2.05 | Topical Treatment With Antagomir-193B-3P Accelerates Diabetic Wound Healing

Jelena Marjanovic, Ivan Jozic, Rivka C. Stone, Cheyanne Head, Beatriz Abdo Abujamra, Robert S. Kirsner, Hadar Lev-Tov, Irena Pastar, Marjana Tomic-Canic

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Background: One of the major contributing factors of healing impairment of diabetic foot ulcer (DFU) is impaired re-epithelialization marked by diminished migration and hyperproliferation of keratinocytes. We used laser capture microdissection (LCM) and genomic approaches to identify specific miRNAs that potentially can contribute to this keratinocyte non-healing phenotype and found microRNA-193b-3p (miR-193b-3p) as one of the top upregulated. Thus, we focused on deciphering the role of miR-193b-3p in epithelialization and therapeutic potential of miR-193b-3p inhibition.

Methods: We collected tissue samples from DFUs (n=15), control skin samples (n=15), and human acute wounds (n=3) and performed LCM and transcriptomic analyses using Ingenuity Pathway Analysis (IPA). qPCR was used to analyze miR-193b-3p and expression of its target genes. We performed gain- and loss-of function experiments. Cell migration was analyzed using scratch assay, and re-epithelialization was evaluated using human ex vivo and diabetic murine in vivo wound models. Formation of stress fibers involved in cell migration was studied using immunocytochemistry and RhoA pull-down assay.

Results: miR-193b-3p was found significantly induced in the epidermis of DFUs, but not in the human acute wounds. Knockdown of miR-193b-3p promoted migration of human keratinocytes, without affecting cell proliferation or cell viability. Moreover, inhibition of miR-193b-3p expression in ex vivo human wounds and diabetic murine in vivo wounds accelerated re-epithelialization. Conversely, overexpression of this miR impaired keratinocyte migration and re-epithelialization in a 3D organotypic wounds. We identified the underlying

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mechanism of miR-193b-3p anti-migratory activity through a disruption of stress fiber formation and a reduction of RhoA activity, with no effect on lamellipodia or filopodia formation. IPA analysis revealed miR-193b-3p target network that contributes to poor healing in DFUs. We confirmed suppression of downstream targets of miR-193b-3p in DFUs (ARHGEF6, CCND1, IGFBP5, JAK2, KIT, KRAS, MAP3K1, NFAT3, SOS2, TCF4, TNS1) and further showed that this transcriptomic landscape found in non-healing DFUs can be reversed upon miR-193b-3p suppression by topical treatment with antagomirs.

Conclusion: We identified miR-193b-3p as an important suppressor of keratinocyte migration in DFUs. Topical treatment with miR-193b-3p antagomiR represents a novel therapeutic approach for promotion of epithelialization in DFUs.

O2.06 | Microfibril Associated Protein 5 in Skin Wound Healing

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[1]Center for Wound Healing and Tissue Regeneration, University of Illinois Chicago, Chicago, IL, United States [2]Department of Histology and Embryology, Rio de Janeiro State University, Rio de Janeiro, Rio de Janeiro State University, Brazil [3]Institute of Clinical Pharmacology, Xiyuan Hospital, China Academy of Chinese Medical Sciences, Beijing, China

Recent studies from our lab demonstrated that fibroblasts are phagocytic in healing skin wounds. RNA sequencing of phagocytic fibroblasts led to the identification of Microfibril associated protein 5 (MFAP5, or microfibril-associated glycoprotein 2/MAGP2) as a molecule that is significantly unregulated in phagocytic fibroblasts. MFAP5, a 25 kD serine and threonine rich small protein, is a component of microfibrils in the extracellular matrix (ECM). MFAP5 is known to be closely associated with fibrosis and angiogenesis in certain cancers. Given the role of MFAP5 in cancer, the aim of this study was to investigate the production of MFAP5 in fibroblasts and in a murine model of skin wound repair. Flow cytometric analysis demonstrated that skin fibroblasts constitutively express MFAP5 and that MFAP5 levels increase markedly in actively phagocytic fibroblasts. Microarray and real time PCR analysis of full thickness excisional wounds showed that MFAP5 mRNA expression is significantly upregulated during the proliferative and remodeling phases of healing (day 3 to 21). Immunohistochemical staining demonstrated that MFAP5 was strongly present in the cytoplasm of dermal cells and in the dermal ECM of scars. In addition, MFAP5 was found to be strongly expressed in human keloids. The data shows that fibroblasts can be a source of MFAP5 in wounds, and that the expression of MFAP5 changes significantly over the course of skin wound healing. The findings support a role for MFAP5 in the regulation of fibroblasts, scar formation, and ECM function in skin wound healing.

Supported by NIH R01 GM50875, R35 GM139603-01, and F31 DE028747-01

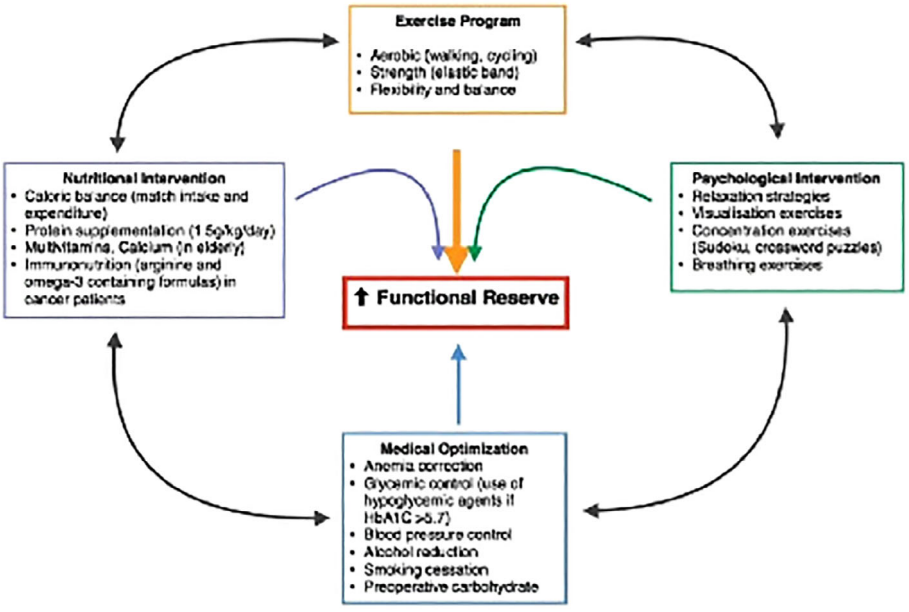
O3: Population and Translational Studies

O3.01 | A Novel Prehabilitation Protocol: Improving Outcomes And Reducing Morbidity In A Complex Surgical Wound Population

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Purpose: Major surgery is associated with a significant decline in functional capacity. While most reconstructive procedures are not considered "major" surgery, some of our procedures do carry significant risks and have associated morbidity. Multiple surgical specialties have begun to implement preoperative patient optimization in an effort to



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reduce postoperative complications. This has been defined as Prehabilitation. Prehabilitation is defined as a series of activities designed to enhancing an individual's functional capacity to enable him or her to withstand a forthcoming stressor, such as surgery. Most prehabilitation programs have moved towards a multimodal approach, including medical optimization, preoperative physical exercise, nutritional support, and stress/anxiety reduction.

Methods: We developed detailed goals for a prehabilitation program including:

-Improving cardiovascular, pulmonary, and/or musculoskeletal function.

-Improve balance and reduce the risk of falls.

-Reduce anxiety and improve coping with specific cognitive behavioral strategies

-Improve quality of sleep with sleep hygiene education.

-Optimize surgical outcomes with smoking cessation interventions.

-Optimize diet with nutrition counseling.

-Educate around postoperative routines to prepare a safe home environment.

Following institutional protocol, we enrolled 65 patients undergoing combination procedures and assigned each of them our prehabilitation protocol (Figure 1). The program was designed to occur over an eight-week period. The patients then underwent their scheduled procedures. Outcome variables including hospital length of stay, re-operation within 30 days, major complications, and minor complications were measured. A historical cohort of similar preoperative morbidity-matched was used as a source for control data.

Results: The results are summarized in the table attached. Significant differences were found in the complications per patient and the number of patients with any complication. Likert scale patient satisfaction also revealed significantly improved scores in patients in the intervention group frequently attributed to "feeling more connected" to their surgeon and office.

Conclusion: Our prehabilitation protocol reduced complications and improved overall satisfaction with combination procedures. While there was almost certainly an increase in preoperative functional capacity in our study patients, this was not discretely measured. As has been well established with enhanced recovery after surgery protocols, a more rapid return to functional capacity after surgery is considered a major factor in complication reduction. With prehabilitation, the patient's preoperative functional capacity is increased. This implies that the physiologic impact of surgery reducing functional capacity results in higher postoperative capacity, and a likely quicker return to baseline capacity.

O3.02 | Racial Disparities In Hidradenitis Suppurativa Management At A Single Center

Nikhi P. Singh[2], Kaitlin Burge[2], Sean Drummond[2], Ryan Zaniewski[2], Spencer MacLeod[2], Lauren Moradi[3], Reagan Hattaway[2], Timothy W. King[1], Tiffany Mayo[4], Jorge de la Torre[5]

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Introduction: Hidradenitis Suppurative (H/S) disproportionately affects African Americans, requiring medical and/or surgical care. Within we describe those affected by this disease at our institution in order to evaluate potential racial disparities.

Methods: IRB exemption (IRB-300006003) was obtained and a retrospective review of 1,187 patients with H/S cared for by plastic surgery and/or dermatology at our institution was performed. Patients were excluded if older than 65. Demographic information, medical history, dermatology treatment history, surgical history, and insurance information was collected. Independent sample t-tests and chi square tests determined statistical significance.

Results: Table 1 illustrates demographics, medical comorbidities, and location of H/S across all cohorts. African Americans were more likely than non-African Americans to be afflicted by H/S of the axilla ($p=0.006$) and groin ($p<0.001$); and were more likely to undergo medical management only ($p<0.001$). White patients were more likely to undergo initial excision surgery ($p=0.001$) and revision surgery ($p<0.001$) and had higher rates of postoperative infection ($p<0.001$) compared to non-white cohorts. There was no difference in the rate of resolution of symptoms across cohorts.

Time from initial presentation to surgery was shorter for African Americans (107 days) than for non-African Americans (162 days) ($p=0.045$). Non-African American patients had a shorter time to follow-up following surgery than African American patients, 4.7 to 6.2 days ($p=0.032$). Non-African American patients had more clinic visits than their African American counterparts, 11 to 6 ($p<0.001$).

Conclusions: African Americans may face disparities in management of this complex and often chronic disease course. Thus, it is important those affected by H/S are managed by a multi-disciplinary team to ensure optimal patient care.

O3.03 | GRANZYME K: An Important Mediator Of Cutaneous Inflammation And Psoriasis

Katlyn Richardson[1], Christopher Turner[1], Hongyan Zhao[1], Angela Burleigh[2], Richard Crawford[1], David Granville[1]

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Background: Granzyme K (GzmK) is a serine protease once believed to induce immune cell-mediated killing. However, recent evidence has challenged this paradigm, suggesting a role for this protease in inflammation. GzmK is abundant in human psoriasis, a cutaneous condition characterized by persistent inflammation and epidermal proliferation forming thick, scaly plaques. The purpose of the present study was to

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assess the role of GzmK in psoriasis. We hypothesized that GzmK contributes to the onset and progression of psoriasis through the augmentation of inflammation and epidermal proliferation.

Methods: GzmK levels and cellular sources were assessed histologically in skin biopsies with and without psoriasis (n=4 each). The role of GzmK was investigated in a murine model of psoriasis, comparing GzmK knockout (K-KO) to wild-type (WT) mice (n=6 per genotype). Psoriasis severity (erythema, squamae) was assessed macroscopically for onset and severity using a modified Psoriasis Area and Severity Index (PASI). Skin tissue extracts were examined for relevant pro-inflammatory markers and epidermal thickness via histology, ELISA, qPCR, and validated in vitro.

Results: GzmK-positive cell numbers were elevated 40-fold (p=0.045) in lesional psoriasis skin compared to healthy skin. Mast cells, macrophages, and neutrophils were identified as the predominant cell sources of GzmK. K-KO mice exhibited an average 60% decrease in psoriasis severity compared to WT mice (scores of 4.25 and 7.25 on 8-point modified PASI scale, p≤0.001 at end of trial). K-KO mice exhibited a reduction by approximately 50% (p=0.0053) in inflammatory cell infiltrate compared to WT mice. Pro-inflammatory cytokines, IL-17A and IL-23, commonly elevated in human psoriasis, were reduced an average of 46% (2147 vs 4657 ρg IL-17A/mg total protein, p=0.044) and 48% (7448 vs 15491 ρg IL-23/mg total protein, p<0.001) in K-KO mice, respectively. K-KO mice also exhibited an average 30% decrease (p=0.014) in epidermal thickness, and 98% decrease (p=0.03) in proliferation markers, compared to WT mice.

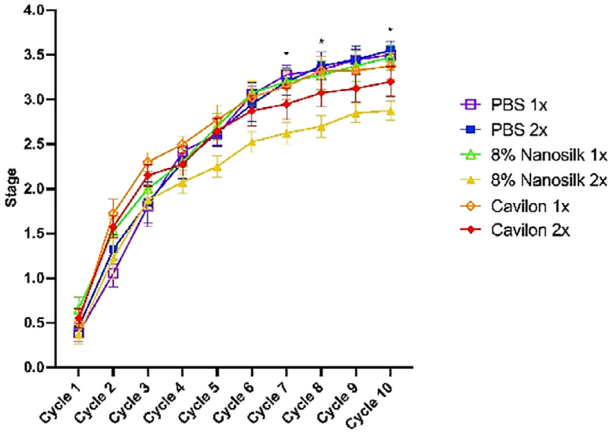
Conclusion: In conclusion, GzmK is elevated in human psoriasis and may contribute to pathogenesis by provoking inflammation and epidermal proliferation. Our combined murine and in vitro human models of psoriasis suggest that GzmK depletion attenuates psoriasis-like skin severity through reduced inflammatory markers IL-17A and IL-23 as well as keratinocyte proliferation.

O3.04 | Nanosilk Spray Prevents The Development Of High Stage Pressure Ulcers

Alyssa E. Vaughn[1], Stephen M. Niemiec[1], James R. Bardill[1], Tanner Lehmann[1], Amanda E. Louiselle[1], Mark A. Bannerman[1], Sudipta Seal[2], Kenneth W. Liechty[1], Carlos Zgheib[1]

[1]Laboratory for Fetal and Regenerative Biology, University of Colorado School of Medicine and Children's Hospital Colorado, Aurora, CO, United States [2]Advanced Materials Processing and Analysis Center, Nanoscience Technology Center, University of Central Florida, Orlando, FL, United States

Background: Pressure ulcers (PU) are chronic wounds associated with significant morbidity and are a substantial burden to the healthcare system. There are limited therapeutics available for prevention and treatment of PU. Silk fibroin is a biocompatible polymer that can be fabricated into nanostructures, termed nanosilk. Nanosilk is characterized by a high strength-to-density ratio and is hypothesized to strengthen the biomechanical properties of skin. We have previously shown that one application of nanosilk reduces the incidence of PU development. Here, we propose that multiple applications of a nanosilk spray would have improved efficacy at preventing PU progression compared to both a control spray as well as Cavilon® spray.



Methods: At 12-13 weeks of age, a 6.35mm diameter steel disk is implanted beneath the external oblique muscle latero-caudally in C57BL/6 mice (n = 59). Mice are given approximately two weeks for the wound to completely heal after implantation. A neodymium bar magnet (6.35 mm diameter, 12.7 mm thick) is applied to the skin superior to the implanted disk for ten, three-hour cycles consisting of two hours with the magnet applied and one hour of rest with the magnet off, with photos taken at the conclusion of each cycle for staging. Six treatment groups were included: one application (1x) of phosphate-buffered saline spray (PBS), two applications (2x) of PBS, 1x and 2x Cavilon® spray, and 1x and 2x 8% nanosilk spray. The first treatment application occurred before cycle one, and the second treatment application before cycle five.

Results: PU development was evaluated using the established National Pressure Ulcer Staging System with grades from 0 to 4. Statistical significance was determined by one-way ANOVA ($p < 0.05$ significant). Two applications of 8% nanosilk significantly decreased the PU stage compared to control, PBS treatment both 1x ($p = 0.03$) and 2x ($p = 0.008$), a difference that achieved statistical significance at cycle seven. Additionally, 2x 8% nanosilk was superior to 1x 8% nanosilk at improving ulcer stage ($p = 0.02$) by the end of 10 cycles. 2x 8% nanosilk also improved ulcer stage compared to 2x Cavilon®, though this did not reach statistical significance.

Conclusion: Two applications of an 8% nanosilk spray was more effective at preventing high stage PU than both control and Cavilon® solutions. Nanosilk shows significant promise as a potential therapeutic for prevention and treatment of PU.

O3.05 | Skin Grafting With Morselized Full Thickness Skin In Recalcitrant Wounds And A Clinical Correlation With A High Viability Full Thickness Morselation Device

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Purpose: The purpose of this study is to assess the efficacy of morselized full thickness skin grafting in difficult to heal wounds

Introduction: Full thickness skin grafts are convenient grafts because the closed donor site makes post-op care easier and less painful. Full thickness skin grafts, by the nature of their thickness and limited donor areas, are not optimal grafts for compromised graft beds or very large wounds. Full thickness grafts that could be morselized into thinner segments and spread out over larger areas would have the benefits of the both the full thickness skin graft donor site and the expandability and thin profile of a split graft. We present a series of patients where full thickness skin grafts are morselized manually with surgical scissors and grafted into full thickness wounds. We also describe a device for morselizing fulling thickness skin to deliver high viability skin morsels for grafting.

Methods: Twenty -Seven patients 13 male, 14 females with non-healing wounds were retrospectively studied. All patients initially received conservative management consisting of dressings and regular debridement. Wounds that failed to progress despite standard of care dressings and were stable for at least 6 weeks and who were candidates for split thickness skin grafting were grafted with manually morselized full thickness grafts. Morselized grafts were treated in the same post procedure protocol as the treatment of a split thickness graft with a non-stick xeroform dressing followed by dry sterile dressing. Pictures were taken at each post visit to document wound healing and wounds were declared healed with complete epithelialization.

Results: Twenty-two patients were followed for the 4 months study period with 20/27 (74%) of patients healed at the end of the follow-up period. Of the 7 non-healed patients 4/6 (67%) of patients had partial graft take and 3 patients had complete graft loss. All patients with some degree of failed grafts had multiple co-morbidities with poor graft bed wound etiologies including radiation, vasculitis, pressure wound, diabetic foot wound, lymphedema, and chronic infection.

Conclusion: These results are consistent with split thickness skin graft results for patients with poor skin graft beds and multiple co-morbidities. Prospective studies with wound type stratification will be needed to further access this approach.

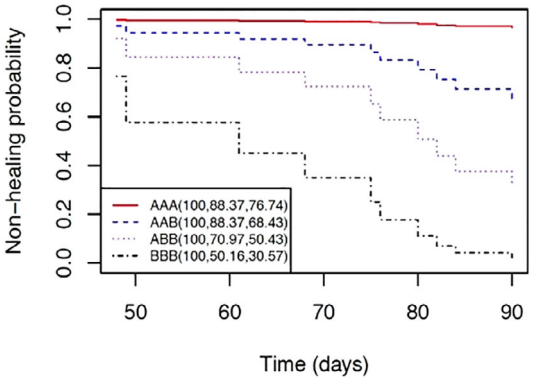
O3.06 | Early Prognosis Of Chronic Wound Healing By Monitoring Wound Alkalinity: A Survivial Analysis

Pei Wang[2], Suvra Pal[2], Jon Senkowsky[3], Shuxin Li[1], Wenjing Hu[1], Liping Tang[1]

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Purpose: The objective of this study was to determine whether wound alkalinity can be used as a monitoring biomarker to prognosticate the healing outcomes of chronic wounds at an early stage.

Methods: Wound alkalinity was monitored by assessing discarded wound fluid-soaked wound dressings, via a disposable and non-contact device - DETEC® pH, from 59 patients at their 1st, 2nd ,3rd, 4th, and 5th visits during routine standard of care.



Non-healing survival curves for change in alkalinity status and non-healing probability during three visits. Numbers (in quote) are estimated non-healing probabilities for each visit.

Results: To identify the relationship between wound alkalinity and wound healing progress, survival analysis was used to investigate the time to complete wound healing. For this purpose, the Cox proportional hazards model with time-dependent variables was used that can accommodate both binary (alkaline or non-alkaline) and continuous (wound area) variables. The outcome is the time to complete wound healing in days, with the event of interest being complete wound healing in 90 days (please see Figure). The overall estimated effect of alkalinity was such that the instantaneous healing rate of a patient with non-alkaline (pH<7) wound was 6.87 times that of a patient with alkaline (pH ≥7) wound. This effect was significant, with a p-value of = 0.008. Furthermore, the overall estimated effect of percentage wound area was such that an increase in wound area by one percentage would reduce the healing rate by a factor of 0.06 (=0.06%). This effect was also significant, with a p-value = 0.0001. Predicted non-healing probability curves were generated to show how the non-healing (or survival) probabilities would change over time for different alkalinity measurements and different values of wound area. Furthermore, just from the first three measurements of alkalinity condition (corresponding to the first three visits), we can predict the non-healing probability pattern for the entire 12-week duration.**Conclusions:** Our results support that the survival model can used to prognosticate the complete wound healing outcome based on the alkalinity measurements during their initial and follow-up visits, and the probability of non-healing will decrease when the wound alkalinity is changed from alkaline to non-alkaline following the initial treatments.

O4: Metabolism and Signaling in Wound Healing

O4.01 | Diet-induced Obesity Dysregulates Rapid Epigenetic Reprogramming To Hypoxia In Macrophages

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Background. Macrophages play key roles in tissue homeostasis and wound healing response. Their functions are dysregulated in the patients with metabolic syndrome such as obesity and diabetes, resulting in poor prognosis of wound healing. Epigenetic reprogramming including histone modification causes macrophage dysregulation in response to external stimuli. The response to hypoxic environment has implications in macrophage functions in inflammation. Whether and how obesity influences histone modification under hypoxic environment is unknown.

Methods and Results. B6 wild-type mice were fed normal diet (ND) or high-fat diet (HFD) for 4-5 months to induce diet-induced obesity. Bone marrow-derived macrophages (BMDMs) were obtained from both groups. BMDMs were treated in hypoxic condition (1% O2) for 1 hour, and the enrichment of histone 3 lysine 4 tri-methylation (H3K4me3) in BMDM was assessed with high-content imaging analysis. We found that hypoxia increases total H3K4me3 levels in BMDMs from ND, whereas this hypoxia-induced H3K4me3 upregulation was abolished in BMDMs from HFD. ChIP-seq was performed to investigate H3K4me3 peaks downregulated in HFD after hypoxia. Interestingly, 79 genes were identified with downregulated H3K4me3 enrichment at the promoter regions in BMDM from HFD compared to ND after hypoxia. As H3K4me3 in the promoter is associated with active transcription, RNA-seq also revealed that hypoxia-induced upregulation was dampened in 42 genes in BMDM from HFD. Gene ontology analysis further revealed 'carbohydrate metabolic process' is associated with the genes identified by multi-omics approach including glycolysis-related genes such as Pgk1, Nupr, and Pfkfb3. Seahorse assay revealed the shift of glycolysis pattern after hypoxia treatment was impaired in HFD BMDM. These results highlight the role of hypoxia-inducible H3K4me3 enrichment in expression of glycolysis-related genes in response to hypoxia and suggest that impairment of this mechanism may cause dysregulation of macrophage metabolism in HFD.

Conclusion. Diet-induced obesity impairs hypoxia-induced shift of glycolysis pattern in macrophages via dysregulated H3K4me3 enrichment followed by transcriptional change of glycolysis-related genes.

O4.02 | Ossabaw Pigs As A Natural Preclinical Model Of Metabolic Syndrome Mediated Impaired Wound Healing

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Background: Infected chronic wounds in the metabolic syndrome (MetSyn) patients represent a major public health burden. The current study recognizes Ossabaw swine as a powerful pre-clinical experimental model to study mechanisms of impaired wound healing under conditions of metabolic syndrome.

Objective: To study wound healing in high fat diet induced MetSyn model of Ossabaw pigs.

Methods: Ossabaw pigs (n=20) were fed either high fat diet (HFD; n=15) or standard chow (n=5) for 8 months. Following 8 months of diet, full thickness (2"x2") excisional wounds were done on the dorsum of the pigs that were infected with 108 cfu/ml of mixed bacterial species of Pseudomonas aeruginosa (PA01), Staphylococcus aureus (USA300) and Acinetobacter baumannii (19606). The infected wounds were followed up to day 31 post-wounding.

Results: The HFD Ossabaw pigs developed MetSyn symptoms including dyslipidemia, abdominal obesity and high blood pressure. In HFD pigs, the wounds associated void space was filled with adipose tissue. Further, they showed increased expression of adipocyte marker perilipin and adiponectin in the granulation tissue. Pigs on HFD showed exaggerated and persistent inflammation in their wounds (n=5, $p<0.05$). HFD pigs showed lower abundance of endothelial cells in the granulation tissue pointing towards impaired vascularization (n=5, $p<0.05$). Masson's trichrome staining of the wound bed showed reduced collagen levels in HFD pigs. Reduced levels of fibroblast marker, FSP1, in HFD pigs pointed towards disorganized granulation tissue formation. The length of rete ridge were significantly reduced in the repaired skin of HFD pigs (n=5, $p<0.05$) predicting compromised biomechanical properties of the repaired skin.

Conclusion: HFD induced MetSyn in Ossabaw pigs with characteristics comparable to clinical outcomes. Specific mechanisms of cutaneous wound healing were compromised in HFD Ossabaw pigs. Thus, this model offers an outstanding opportunity to study the cellular and molecular bases of healing outcomes in metabolic syndrome like obesity.

O4.03 | Systems Biology Approaches To Understanding Mechanisms Of Chronic Wound Progression

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Our previous studies showed that gene expression in cutaneous chronic wounds differs significantly from non-chronic wounds in the first 48 hours post-wounding. Here, we performed an RNASeq analysis using tissue from both non-chronic and chronic wounds to study progression of chronicity. We collected tissues from early stages of chronic wound development (mild chronicity), and when the wound was chronic (full chronicity). A Weighted Gene Correlation Network Analysis (WGCNA) was performed in R on 21919 genes identified in the RNAseq using Kallisto. WGCNA segregated the 21919 genes into 14 modules and also found modules significantly correlated ($p<0.05$) to mild and full chronicity. Genes belonging to these modules were used for a pathway analysis using pathfindR. Modules significantly correlated to mild chronicity showed enrichment of several pathways. The 'Ephrin mediated growth cone collapse' pathway, leads to poor healing of motor and sensory neurons which often accompany chronic wounds. The 'ErbB signaling' pathway showed that various ligands that bind to EGFR were dysregulated in chronic wounds. For example, Hbegf is highly upregulated in healing wounds

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but in chronic wounds Hbegf is not upregulated. Application of rHB-EGF restores re-epithelialization in mice. The 'Focal adhesion' pathway showed dysregulated integrins and laminins, molecules that are important in proliferation and migration of fibroblasts and endothelial cells. Finally, in mild chronicity, several pathways were related to fibroblast/myofibroblast dysfunction. The major gene involved in this dysfunction is alpha-smooth muscle actin (Acta1) which was upregulated in chronic wounds. Acta1 overexpression hampers proliferation and fibroblast-mediated repair. In full chronicity, the 'JAK-STAT signaling' pathway was suppressed, a finding that indicates similarity to melanoma development, and undermines the immune system impairing defenses against microbes. Furthermore, the pathway 'Syndecan interactions' was enriched with the gene Sdc1 overexpressed in chronic wounds. In melanoma cells Sdc1 overexpression modulates MMP9 to enhance metastasis. Moreover, the 'Neuroactive ligand receptor interaction' pathway was enriched and Adra2b was overexpressed which downregulates cAMP. Applying cAMP analog to open wounds improved re-epithelialization and granulation tissue formation. In conclusion, our studies show that during development of chronicity, numerous cell and molecular signaling pathways involved in arresting healing are present, and that there are parallels between the processes involved in chronic wounds and melanoma. Testing these pathways in vivo, could lead to the discovery of candidates for treatment of chronic wounds.

O4.04 | Fibronectin-Derived Peptide P12 Promotes In Vitro And In Vivo Angiogenesis

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Background: Cyclized P12 (cP12), administered intravenously (iv) at 0.01mg/kg, 1-4h post-burn, limits burn injury progression, speeds healing, and reduces scarring in our porcine vertical injury progression burn model (WRR 24:501-513, 2016).

Study Goal: 1. To determine whether P12 promotes cultured human endothelial cells (HEC) growth and in vitro angiogenesis. 2. To determine whether P12 in an angio-reactor mouse model stimulates in vivo angiogenesis.

Method: For studies on angiogenesis we used three established methods:

1. XTT assay to determine HEC growth on collagen-coated plates
2. An in vitro angiogenesis assay using HEC cultured on collagen-coated microbeads that were dispersed in a human fibrin gel (Feng, et al. Int J Cell Biol, 13:231-279, 2013).
3. An in vivo angiogenesis assay using nude mice and a commercially available assay kit containing angioreactors and matrix for implanting into the flanks of the mice. Although the kit contained MatrigelTM as a hydrogel delivery system for potential angiogenesis factors, we found that human fibrin gel for delivery of peptides was superior.

Results: P12 increased angiogenesis both in vitro and in vivo.

1. In endothelial cell defined medium with or without vascular endothelial cell growth factor (VEGF), P12 stimulated HDMEC growth in a dose response manner from 3-30uM without VEGF or with 1 or 50ng/ml VEGF. P12 stimulated optimal HDMEC growth when 10uM P12 was added to cultures containing 50ng/ml VEGF.
2. The in vitro angiogenesis assay demonstrated that 3uM P12 with VEGF and Fibroblast Growth Factor-2 (FGF-2) at suboptimal doses (15 ug/ml and 12.5 ug/ml, respectively) doubled sprout angiogenesis from EC-covered beads compared to no P12 under the same conditions.
3. The in vivo angiogenesis assay demonstrated that the addition of 10 or 30uM cP12 to a low dose of FGF-2 (50ng/ml) in fibrin gel increased angiogenesis 7-fold and 15-fold compared to no cP12. Similarly, 10 or 30uM cP12 addition to a low dose of VEGF (125ng/ml) in fibrin gel increased angiogenesis 80% and 130% compared to control.

Conclusions: P12, a fibronectin-derived peptide, promotes HEC growth and stimulates angiogenesis by enhancing the effect of angiogenic growth factors in vitro and in vivo.

O4.05 | Accelerated Wound Closure In Diabetic Mice Mediated By Extracellular Vesicles With A Defined Protein Payload

Dong Jun Park[1], Erika Duggan[2], John Nolan[2], Brian P. Eliceiri[1]
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Impaired wound healing in diabetic patients is a major clinical problem in the US, affecting 25% of the diabetic population in their lifetime. Dysregulation of endogenous intercellular communications between stromal and immune cells in the wound bed is hypothesized as the basis for impaired vs. normal wound healing. Here, we focus on the role of extracellular vesicles (EVs) as mediators of these intercellular communications and identify candidate EV protein payloads that distinguish EVs in the wound bed of a mouse model of diabetic (db/db) vs. wildtype (WT) mice. First, we establish an approach for the culture and collection of EVs from a donor site that can be used for adoptive transfer to a recipient wound site. These donor EVs are collected from subcutaneous polyvinyl alcohol sponges of db/db or WT mice, and used to demonstrate that the WT donor EVs promote accelerated wound closure in db/db recipients compared to EVs from db/db donors. These studies define conditions in which WT EVs are sufficient to increase closure in an impaired model of wound healing. Second, we subject EVs from db/db vs WT mice to an unbiased analysis of protein payload using mass spectrometry and identify a hypermetabolic protein profile and evidence to support an impaired remodeling of the extracellular matrix in db/db vs. WT mouse EVs. Finally, we report on a lentiviral-mediated gene delivery system of a reporter fusion construct whereby green fluorescent protein (GFP) is expressed inside an EV

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to follow trafficking and uptake. With the demonstration that EVs from the WT and db/db wound are distinct on the basis of the biological activity and protein payload, we have a candidate 'hit' list and a delivery strategy to test their efficacy and uptake in the wound healing response.

O4.06 | Human And Mouse Chronic Wounds Share Common Signaling Pathways

Proma Basu, Manuela Martins-Green
Molecular Cell and Systems Biology, University of California, Riverside, CA, United States

We have previously shown that our murine model of chronic wounds has many characteristics of human chronic wounds. In this study we have compared the data gathered from our mouse model with chronic wounds to that collected from human chronic wounds, to understand the similarities and discover important biomarkers for wound chronicity as well as identify candidates for treatment. We compared our RNAseq analysis to that obtained in human chronic wounds in the last 10 years. Three transcriptomic studies that compared human diabetic chronic wounds to either diabetic or non-diabetic skin were identified. One bulk RNASeq and two single cell transcriptomic studies were identified for comparison. Comparison to the bulk RNASeq study done by Ramirez et al. (2018), identified 318 genes which are significantly differentially expressed (FDR<0.05) and show a similar trend of log fold change in expression in humans and mice chronic wounds. A Reactome pathway analysis showed a prevalence of pathways related to 'EGFR signaling'. Our data indicate that SHC1 and GRB2 mediated stimulation of EGFR activates mitogenic pathways downstream via tgfa, epiregulin, proepiregulin and areg. This overstimulated EGFR signaling is a known biomarker of oncogenic processes. The single cell transcriptomic study published by Theocharidis et al. (2020) identified 206 similarly expressed genes. Overexpression of mmp3, mmp14 and ctsk explained the enrichment of the pathway 'Degradation of ECM'. Additionally, 'Syndecan interactions' was enriched and consisted of fn1, col1a1 and postn, all overexpressed. The upregulation of all three genes is a hallmark of tumor growth and metastasis in melanoma and breast cancer. Postn overexpression in particular reduces cell adhesion augmented by COL1 and FN1 and facilitates metastasis in humans. The study by Januszyk et al. (2020), identified 24 genes clustering significantly in fibroblasts, of those, 22 genes were common with mouse chronic wounds. The pathway with the highest fold enrichment was 'Activation of AP-1 family of transcription factors'. Both jun and fos, which form AP1, were overexpressed in chronic wounds. Again, the pathway 'Syndecan interactions' was enriched and consisted of the genes fgf2, col1a1 and col3a1which were all overexpressed. Fgf2 overexpression is linked to tumor progression in melanoma. In conclusion, these studies substantiate the efficacy of our mouse model for chronicity studies and provide biomarkers to identify candidates for proof-of-concept studies in humans.

2022 WHS Poster Abstracts

IRD POSTERS

IRD1. | Biodegradable Compositions With Novel Antimicrobial Peptide For Managing Wound Infections

Jennifer Neff[1], Danir Bayramov[1], Esha Patel[2], Nirav Patel[1], Hsiaofong Lu[1]
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Purpose: Develop a stable, biodegradable wound treatment comprising a novel antimicrobial peptide, ASP2, and demonstrate efficacy against biofilms of multi-drug resistant (MDR) organisms grown on porcine skin ex vivo.

Methods: Chitosan sponges (1 cm in diameter and 0.5 mL volume) containing 2.5 mg ASP2 were prepared by lyophilizing liquid formulations at -50 oC.

Efficacy Evaluation in Ex Vivo Porcine Skin Biofilm Model: Porcine skin samples, 9 mm in diameter, were incubated in 0.5 mL bacterial culture (106 CFU/mL of Methicillin resistant Staphylococcus aureus, USA 300 ATCC BAA-1717 (MRSA) or Pseudomonas aeruginosa ATCC 15692 (P. aeruginosa) at 37°C for 72 hours for biofilm formation. Skin samples with adhered biofilms were then treated with ASP2 chitosan sponges in 0.2 mL of 3% albumin or 50% porcine serum at 37oC for 24-72 hrs. After 1, 2 and 3 days, bacteria were recovered from the skin by sonicating in D/E neutralizing broth and plating on TSB/agar. Controls were treated with saline and tests were performed in triplicate.

Stability Post Sterilization: ASP2 sponges were sealed in aluminum foil pouches containing an oxygen-absorbing sachet and subjected to E-beam sterilization at 35 kGy. ASP2 content and impurity were measured in triplicate samples by RP-HPLC immediately post sterilization and after storage for 1, 3, 6, 9, 12 and 18 months at room temperature. Unsterilized samples served as controls.

Results: When wetted, ASP2 chitosan sponges formed gels. After a single sponge treatment, 2-3 log CFU reductions were achieved in both MRSA and P. aeruginosa biofilms by 24 hours, followed by 3-5 log CFU reductions after the 2nd day, and complete or nearly complete eradication (~ 6 log CFU) after the 3rd day post-treatment relative to saline controls. Bacteria reductions were significant at all time points (t-test, $p < 0.05$).

Minimal ASP2 degradation was observed post sterilization; after 18 months of storage, ASP2 impurities increased by 0.4% and 0.8% for non-sterilized and sterilized samples, respectively.

Conclusions: Biodegradable sponges comprising the broad-spectrum antimicrobial peptide, ASP2, display potent activity against MRSA and P. aeruginosa biofilms grown ex vivo on porcine skin and exhibit long term stability post-sterilization. Future studies will evaluate ASP2 treatment toxicology, wound healing and efficacy in porcine models of excisional and burn wound infection.

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IRD2. | Skin Strain Modeling To Assess Removal Of Acrylate Vs Hybrid Negative Pressure Wound Therapy Drapes

Amy K. McNulty, Robert Wilkes, Jason Bjork, Michael Turnbull, James Sieracki

3M Company, St. Paul, MN, United States

Background: Negative pressure wound therapy (NPWT) drape removal from skin may be painful for patients and inadvertently cause skin damage during the length of therapy. Most NPWT drapes utilize an acrylate adhesive to achieve the seal. To improve the experience associated with NPWT drape removal, a novel hybrid drape was developed. This drape is composed of areas of acrylate adhesive and areas of silicone adhesive. To more fully understand how removal of the hybrid drape versus the acrylate drape affects skin, adhesive removal models have been developed. Existing medical adhesive removal test methods include measuring peel forces during medical product removal from steel, acrylic resin products, or skin. However, the peel force may not be representative of the actual strain that the product imparts to tissue. The current study assessed the differences in strain profiles for acrylate versus hybrid NPWT drapes using a finite element analysis (FEA) to measure strain and deformation that occurs at the tissue interface with the NPWT drape.

Methods: A skin mimic was created from a two-part silicone molding material. The surface of the skin mimic was a polyurethane film. Three NPWT drapes were tested: one acrylate adhesive* drape and two hybrid acrylate plus silicone adhesive drapes (hybrid drape 1 and hybrid drape 2). The drapes were placed on the skin mimic and a high-speed camera captured the NPWT drape removal. The 180-degree peel force was captured at the same time. FEA models of the drapes and drape removal were based on mechanical test data of the drapes as well as the peel force data.

Results: The FEA modeling showed that the maximum principal strain associated with removal of the acrylate drape was 47.3%, whereas the maximum principal strain associated with the removal of hybrid drape 1 was 21.5% and hybrid drape 2 was 16.1%. The average peel force associated with the acrylate drape was 66.1 gf/in, hybrid drape 1 was 112.5 gf/in, and the hybrid drape 2 was 92.2 gf/in. The hybrid adhesive drape had the highest peel force and the lowest skin strain.

Conclusions: NPWT drape removal may, in certain instances, be related to pain and periwound skin injury. This hybrid drape may provide clinicians an option for NPWT that is gentle to skin.

*3M™ V.A.C.® Drape (3M, Maplewood, MN)

IRD3. | Assessing The Gentleness Of A New Hybrid Drape Compared To Traditional Acrylate Drape

Amy K. McNulty[1], Michael Turnbull[1], Michele Anderson[2], Anna Bailey[1], Dan Morse[2], Iwen Grigsby[1]

[1]MSD, 3M Company, St. Paul, MN, United States [2]3M Company, St. Paul, MN, United States

Introduction: Key biological functions of the stratum corneum include a first line of defense against environmental insults and pathogens, regulation of hydration, exclusion of external toxins and allergens, and initiation of repair processes.1 To help preserve integrity of periwound skin and improve patient experience with negative pressure wound therapy, a novel hybrid drape was developed. This study assesses the impact of repeated placements and removal of hybrid drape compared to traditional acrylate drape on the volar surface of the forearm.

Methods: This study was performed using an institutional research board approved protocol. Strips of hybrid drape* or acrylate drape** (1 in x 3 in) were placed on each volar forearm of 44 healthy humans. Drape strips were removed and replaced with new strips every 2 – 3 days for a total of 5 times. Transepidermal water loss (TEWL) was measured at baseline and after every drape removal. Drapes from the first and fifth removal were saved and stored at -80°C. A protein assay kit was utilized for the bicinchoninic acid (BCA) and IL-1a analysis. BCA analysis was performed to determine quantity of protein removed from the skin after the first and fifth drape removal and IL-1a (an inflammatory cytokine) was measured using the fifth drape. Subject reported pain upon dressing removal was assessed using a 100 mm long visual analogue scale.

Results: The change from baseline TEWL to TEWL after the 5th removal was higher for the acrylate drape than for the hybrid drape ($p<0.05$). The hybrid drape removed less total protein than acrylate drape at the fifth drape removal ($p<0.0001$). Post-hoc analysis showed significantly less IL-1a generated following the fifth drape removal for the hybrid drape ($p<0.0001$). Post-hoc analysis also showed that subject reported pain was significantly higher with the acrylate drape at all drape removals ($p<0.001$).

Discussion: The hybrid drape was shown to be gentler to the skin than the traditional acrylate drape with respect to changes in barrier function as measured by TEWL, decreased protein removal, decreased production of the inflammatory cytokine IL-1a, and decreased pain upon removal.

*3M™ Dermatac™ Drape, **3M™ V.A.C.® Drape, 3M, St. Paul, MN

IRD4. | Treatment of Diabetic Foot Ulcers Using Autologous Adipose Tissue

Ahmed N. Zobi

Syntr Health Technologies, Inc., IRVINE, CA, United States

Background: Diabetic foot ulcer (DFU) is a complication of diabetes mellitus (DM) and the leading cause of nontraumatic lower limb amputations. The lifetime incidence of DFU formation in individuals with DM may be as high as 25%. In 2013, an estimated 384 million individuals suffered from DM . The etiology of DFU is multifactorial and primarily includes some combination of neuropathy, poor vascularity, and isolated or repeated trauma. Once a DFU has formed, the wound microenvironment is characterized by poor healing due to continued pressure and/or trauma, chronic infections, and displacement of the

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plantar fat pad. Current DFU treatments are laden with expense and unpredictable outcomes; however, emerging evidence indicates that autologous microsized fat tissue may be a safe and effective alternative to the current treatment options.

Methods: In this randomized controlled trial with two arms, 28 patients will be randomized to standard of care or to microsized adipose tissue injection prepared using the SyntrFugeTM System, which will output a minimally manipulated adipose tissue. Clinical outcomes were determined weekly up to 12 weeks and then 2-4 week post wound closure. Primary Endpoints is calculating the heal rates of the subjects treated with the adipose tissue processed with the SyntrFuge™ System versus Control (SOC). As well as obtaining the wound closure data at a 12 week timeframe. Secondary Endpoints will included Percent Area Reduction (PAR) at 4, 8 and 12 weeks, Safety in relation to the number of adverse events (all types) reported while the device is in use against the control group.

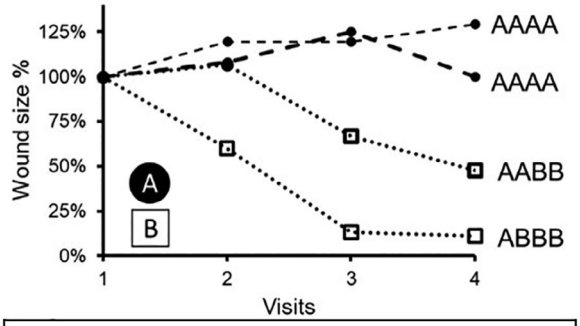
Preliminary **Results:** Initial recruitment for this study has/is currently following 3 patients. One patient that has undergone the SyntrFuge Treatment arm of the study has completed healed and closed their wound in under 3 weeks. The patient started off with a 1.3cm2 wound and decreased to .1cm2 in just one week and continued 2 further follow-up visits until week 3 when 100% of the wound closed. The 2 other patients in the SOC group have yet to achieve full woundc closure to date (4 weeks into the study). We will continue to study the patients and recruit more in the next few months. No treatment-related adverse events nor relapses were documented.

Preliminary Conclusion: With this first-in-human data, we understand that more data is needed. We believe that that over the course of the next 2-3 months we should complete a good portion if not all the patients needed for our 28 patient randomized controlled study. However, this initial human data showing complete wound closure at 3 weeks post a single injection of microsized adipose tissue processed with the SyntrFuge system is very pormising. The SyntrFuge system overcomes manual processing techniques related to the processing of adipose tissue, along with adding the benifits of standardization and automation of the process to output consistent microsized adipose tissue for the treatment of DFUs.

IRD5. | Early Assessing Hyperbacric Oxygen Therapy Efficacy By Monitoring The Change Of Wound Alkalinity

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Purpose: The aim of this study was to determine whether wound alkalinity can be used as a monitoring biomarker to assess the effectiveness of hyperbaric oxygen (HBO) therapy on diabetic foot ulcers (DFUs).



Changes of wound size percentage for representative HBO treated patients. The wounds with a change of A to B were associated with a better wound size reduction compared to those remain alkaline. (AB vs AA)

Methods: Wound alkalinity was monitored by assessing discarded wound fluid-soaked wound dressings, via a disposable and non-contact device - DETEC® pH, from 16 DFU patients who have been taking continuous and indistinctive HBO treatment. The wound alkalinity at the 1st, 2nd, 3rd and 4th visits during the treatment period was correlated with the subsequent week's wound size reduction rate.

Results: The results of 16 patients with 103 alkalinities and wound size measurements were collected and used in these analyses. By plotting line chart of wound size change over visit times (please see Figure), patients who had a wound alkalinity changing from alkaline (pH ≥ 7.0) to non-alkaline (pH < 7.0) were found with significantly larger wound size reduction than those who remained alkaline (p=0.0931 for the 3rd visit, and p=0.0266 for the 4th visit). The change of wound alkalinity (from alkaline to non-alkaline) may show the wound environment changes, perhaps the increase in inflammatory responses, which then led to increased wound size reduction. To investigate whether continuous wound alkalinity determination would help to assess HBO treatment effectiveness, the data was used to build a linear mixed effect model in which wound alkalinity was input as a fixed main effect and random effects of dependent repeated wound healing rates corresponding to the same patient and individual variations from different patients were also added. The analyses show that, under the repeated measures setup, "wound alkalinity" is a significant factor of wound healing rate (p=0.0909). The estimated coefficient corresponding to non-alkaline wound turned out to be positive (0.018499), which implies that the wound reduction rate increases when wound milieu is continuously at "non-alkaline" state.

Conclusions: The results support that wound alkalinity may be used as a monitoring biomarker to provide quick effectiveness assessment of HBO treatment in DFUs.

IRD6. | A Non-Contact Device For Fast Screening Of Infections In Diabetic Foot Ulcer And Venous Leg Ulcer

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Purpose: Infection is the most likely single cause of delayed healing in chronic wounds. Recent study shows that infected wounds have elevated leukocyte esterase activities as compared with non-infected wounds as determined by a portable device - Detec® Esterase. Here we investigated whether the same device, Detec® Esterase, can be used to screen and diagnose the infection of diabetic foot ulcer (DFU) and venous leg ulcer (VLU) at point-of-care.

Method: Wound dressings from 71 DFU patients and 31 VLU patients 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: For DFU patients, the results show that DETEC® Esterase device had good sensitivity (89.8%), fair specificity (45.5%), moderate accuracy (76.1%), and the increase in post-test risk of infection with positive test result of 9.56%. As for VLU patients, the results show that DETEC® Esterase device had comparably good sensitivity (82.1%), moderate specificity (66.7%), good accuracy (80.6%), and a smaller increase in post-test risk of infection with a positive test result of 5.51% because of high prevalence of the condition. Through fisher's exact test to compare the diagnosis efficiency between DFU and VLU, two tailed p-values for sensitivity, specificity, and accuracy are 0.4825, 0.593, and 0.7975, respectively. Therefore, there are no significant differences in the device diagnosis efficiency between two different wounds. Using a logistic regression model, we determined whether other factors (such as age, gender, race, initial wound size, wound location, and sign of infection) would affect the accuracy of DETEC® Esterase device on diagnosis of infection and found none of these factors has significant effect on diagnosis accuracy of DETEC® Esterase device (age, P=0.4597; gender, P=0.1886; race, P=02401; initial wound size, P=0.9020; wound location, P=0.8549; signs of infection, P=0.456).

Conclusion: Our results support that DETEC® esterase can effectively and accurately diagnose and/or screen for infections in DFUs and VLUs. The diagnosis accuracy of the device is not influenced by the patients' age, gender, race, initial wound size, wound location, and sign of infection.

IRD7. | Encapsulation of MSCs in Self-Assembling Peptide Hydrogels with Activity Against Methicillin-Resistant Staphylococcus aureus (MRSA) and Pseudomonas aeruginosa (Pa) Promotes Faster Wound Closure and Improves Tissue Regeneration in a Clean and Pathogen-Contaminated Type I Diabetic (STZ-induced) Mouse Model

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About one-fifth of diabetes-related hospitalizations are the result of complications from delayed wound healing and pathogen colonization, leading to ~100,000 non-trauma-induced lower-limb amputations per year and ~35,000 deaths in the US each year due to the rise of antibiotic-resistant strains of bacteria. The objective of this research is to develop a flowable cell delivery scaffold to promote tissue regeneration in clean and pathogen-contaminated diabetic wounds.

Hypothesis: This shear-thinning-self-assembling-peptide gel matrix (G4matrix) encapsulated with mesenchymal stem cells (MSCs) enhances implanted cell survival and promotes healing in both clean and pathogen-contaminated diabetic wounds vs. control (PBS + MSCs).

Methods: 5-mm full-thickness, splinted, excisional wounds (n= 50 mouse, 2 wounds/mouse) are created on the dorsum of each mouse. Tissues were collected at days 2 and 21 post-wounding for further analysis.

Results: The G4matrix used in this research are effective against Pa and MRSA. We demonstrated complete clearance of 106 CFU (MRSA) and complete clearance of 106 CFU (Pa) which was significantly ($p<0.0001$) higher than a non-antimicrobial matrix control. To confirm cell viability, we encapsulated MSCs in G4 matrices for 24 hours and used a luciferase-based assay to quantify cell viability using Relative Luminescence Units (RLU). We demonstrated significantly higher cell viability in G4matrix ($p<0.0001$) compared to gelatin, our positive control, and PBS. MSCs encapsulated in G4matrix resulted in significant ($p < 0.02$) reduction in the wound area of non-contaminated wounds at day 21 versus control (PBS + MSCs). Similarly, at day 21, wounds were found to have complete healing with immature epithelium over the center of the wounds for clean wounds. Furthermore, H&E staining of sectioned wound tissue from pathogen-contaminated wounds demonstrated that the wounds treated with G4matrix + MSCs have robust granulation tissue and re-epithelialization with significantly lower edema ($p <0.001$) at days 2 and 21, and significantly lower leukocyte infiltrate levels ($p < 0.02$) at days 2 vs. controls.

Conclusion: G4matrix + MSCs improved wound closure in non-contaminated and decreased markers of inflammation in contaminated wounds as compared to untreated controls.

Biomaterials In Wound Healing And Tissue Regeneration

P1. | 3D Cell Printed Pre-Vascularized Muscle Tissue For Functional Muscle Recovery In Rat Model

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Aims: Volumetric muscle loss (VML) is an irrecoverable injury generally more than 20% muscle loss. The decellularized extracellular matrix (dECM) approach has been extensively investigated. In this study, we

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developed pre-vascularized muscle tissue constructs for VML treatment using muscle dECM (mdECM) bioink through 3D cell printing. The regenerative capability of muscle tissue constructs was evaluated through implantation in a rat model of the VML defect.

Methods: Human muscle cells and human umbilical vein endothelial cells (HUVECs) were encapsulated in mdECM and vascular-derived dECM (vdECM) bioink. Pre-vascularized skeletal muscle constructs were fabricated in the gelatin granule reservoir. To create the models of VML injuries, 10-week-old Sprague Dawley rats were anesthetized, and 40% of the tibialis anterior muscle was removed from each rat. The 3D cell printed muscle constructs were implanted and sutured at both ends. Masson's Trichrome staining, immunofluorescence staining, and functional assessment were conducted at four weeks post-impanation.

Results: In vitro assessments showed that the pre-vascularized muscle tissue construct that was formed by a coaxial nozzle (coaxial group) exhibited enhanced contractile force and myotube and endothelial network formation. In vivo results revealed that the muscles treated with the coaxial group greatly improved de novo muscle fiber regeneration, vascularization, and innervation. Most importantly, the pre-vascularized muscle tissue construct achieved 85% functional recovery in VML injuries.

Conclusions: In this study, we developed a pre-vascularized muscle construct using 3D cell printing and a coaxial nozzle. We expect that these results will provide a blueprint for the future development of engineered human-scaled muscle tissues for VML injury treatment.

P2. | Successful Outcomes Using Aseptically Processed Human Reticular Acellular Dermal Matrices In Treating Premature Neonatal Patients With Complex Wounds

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Introduction: Premature infants pose numerous concerns when addressing wounds that arise in the neonatal intensive care unit (NICU). The immaturity of their skin, the lack of an innate immune system to protect from infections, and the use of advanced wound dressings that are often designed for adult patients are common barriers to effective wound healing. Identifying safe and effective dressings to treat complex wounds in this fragile population is often a challenge for wound care specialists. The purpose of this study was to evaluate the safety and efficacy of human reticular acellular dermal matrices (HR-ADM) on large complex wounds in neonatal premature patients. Such wounds are often at risk for contractures and scars if improperly treated. Long-term complications from improperly treated wounds may result in limitations in range of motion.

Methods: Premature infants (< 37 weeks gestation) hospitalized within the NICU with complex wounds were chosen to receive the aseptically processed HR-ADM. Four patients are illustrated in this case series ranging from 23 -33 weeks gestation. Three patients suffered severe IV extravasation injuries and one suffered from multiple traumatic skin tears. The allograft tissue was applied at the bedside over healthy wound beds debrided of nonviable tissue, covered with non-adherent dressings, and left in place for seven days without disturbing the underlying graft. After one week, the wounds were examined.

Results: In each case, a single application resulted in successful granulation and closure of the open wounds within 10-14 days. No complications, infections, or side effects were encountered.

Discussion: This case series illustrates the safety and efficacy of an aseptically processed human reticular acellular dermal allograft for use within the premature neonatal population. The reticular dermal allograft was easily applied at the bedside and wounds rapidly healed after a single application. No contractures developed and residual scars were minimal.

P3. | Engineered Nanotrap Hydrogel Sequester Local Inflammatary Mediators And Deliver Antibiotics To Facilitate Chronic Wound Healing

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Background: Chronic wounds cause great burden to both patients and society, with the impact still rising as the general population continues to age and prevalence of chronic diseases (diabetes, cardiovascular disease) increases. Despite of the heterogeneous etiology, most chronic wounds share common features, including prolonged and excessive inflammation, and presence of bacterial infection. Therefore, attenuation of proinflammatory factors, and sustained local delivery of antibiotics are considered as viable approaches to treat chronic wounds. We have developed a versatile telodendrimer (TD) nanoplatform, called nanotrap (NT) for efficient binding of protein or small molecule drugs via the combination of multivalent and synergistic charge and hydrophobic interactions.

Methods: The conjugation of TD NT onto microporous polyethelene glycol (PEGA) based hydrogel resins allows for topical application. Via rational selection of charge moiety and fine-tuning of NT density/valency, the NT resins can be engineered in a customizable fasion for protein adsorption and drug release. Herein we hypothesize that optimally engineered NT resin can improve chronic wound healing outcomes by attenuating proinflammatory cytokines and infection control.

Results: In vitro results show the engineered resin demonstrates higher protein loading capacity and superior proinflammatory cytokine removal efficiency (n=3). In vivo preliminary results using HLL mice, a luciferase reporter for NF-κB activation and inflammation responses, shows optimized NT resin demonstrated significant efficacy in attenuating inflammation. Our results also show antibiotics polymyxin B (PMB) loaded in NT resin can be sustainably released out and exert comparable antimicrobial activity as free PMB, evidenced by the Minimum Inhibitory Concentration (MIC) assay. Using animal model with E.coli bacterial wound infection, we demonstrated that the application of PMB-loaded hydrogel significantly reduced the severity of

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infection-induced ulcerative skin compared to the untreated infectious wounds (n=3). In addition, no toxicity is observed when the NT hydrogel were co-incubated with RAW 264.7 cell line (n=3).

Conclusion: Overall, these results suggest the application of optimized TD hydrogel resin has the potential to attenuate proinflammatory cytokines and control infection in local wound immune milieu to facilitate the healing of the chronic wounds.

P4. | Viable and Devitalized Amnion Membranes Promote Wound Granulation In A Delayed Murine Excisional Wound Model

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Purpose: Chronic or delayed healing wounds constitute an ever-increasing burden on healthcare providers and patients alike. Thus, therapeutic modalities that are tailored to particular deficiencies in the delayed wound healing response are of critical importance to improve clinical outcomes. Amnion-derived viable and devitalized allografts have demonstrated clinical efficacy in promoting the closure of delayed healing wounds, but the mechanisms responsible for this efficacy and the specific wound healing processes modulated by these products are not fully understood.

Methods: Here, we utilize a diabetic murine excisional wound model in which healing is delayed by splinting, and we applied viable amnion membrane allograft (vHAMA) or devitalized amnion membrane allograft (dHAMA) to the wound bed in order to determine their effects on wound healing processes.

Results: Compared to control wounds that were allowed to heal in the absence of treatment, wounds to which vHAMA or dHAMA was applied demonstrated enhanced deposition of granulation tissue accompanied by increased cellular proliferation and increased de novo angiogenesis. In addition, vHAMA-treated wounds also demonstrated accelerated re-epithelialization.

Conclusions: Taken together, these data suggest that both dHAMA and vHAMA facilitate delayed wound healing through promoting processes critical to granulation tissue formation. Further understanding of the cellular and tissue mechanisms underlying the effects of matrix products on wound healing will enable tailored prescription of their use in order to maximize clinical benefit.

P5. | Tissue Regenerative Devices with Allogeneic Cells – A Critical Review

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Introduction: The overall objective of this technical review is to highlight the potential health risks of incorporating living allogeneic cells (like fibroblasts, keratinocytes1, primordial somatic stem cells2, bone marrow mesenchymal stromal cells3, etc.) in skin substitutes or wound healing devices for tissue regeneration. Please make a note our emphasis is only on allogeneic cells and not of autogenic origin. At the end of this review, you may be convinced the usage of allogeneic cells may have to be limited or restricted in clinical usage. From the Biomaterial Science perspective, the main focus of this publication is to reveal the biological safety of allogeneic cell incorporated biomaterials.

Method: A considerable amount of literature study was accomplished to fulfill the required methodology for this review article. Accordingly, we have focused on varied scientific publications about the rejection phenomenon of allogeneic cells in the host tissue. We also foresee the impact of our observation towards the FDA regulations to assess the safety and efficacy of such devices containing allogeneic cells and molecules. Additionally, this would help to seek the eligibility of reimbursement valuation by Medicare or other insurance entities.

Results & **Conclusion:** The cellular and molecular pathways of the immune response are recently being better understood. From the clinical perspective, the presence of allogeneic somatic stem cells in a skin substitute is quite objectionable due to possible immunogenicity to the host tissue. Several evidences4,5,6 have clearly documented the rejection of allogeneic cells in the host body. Adding fuel to fire, the additional drawback that many fail to focus on is the presence of elastin molecules in such constructs like amnion/placenta/umbilical cord, etc.

Recently, more light has been thrown on the potential carcinogenicity of elastin due to its biodegraded byproduct peptides7. It is well-known that fibroblast is responsible for the secretion of elastin8. In the case of allogeneic stem cell implantation, it is evident that the stem cell-derived fibroblast in the host tissue will secrete elastin molecules native to the donor fibroblasts. Naturally, the recipient body degrades the donor's elastin releasing the carcinogenic byproduct, elastokines.

References:

1. https://www.frontiersin.org/articles/10.3389/fbioe.2018.00086/full
2. https://pubmed.ncbi.nlm.nih.gov/23154338/
3. https://pubmed.ncbi.nlm.nih.gov/30651054/
4. https://royalsocietypublishing.org/doi/10.1098/rsif.2009.0403
5. https://stemcellres.biomedcentral.com/articles/10.1186/s13287-019-1203-3

Cell-Cell and Cell-ECM Interactions In Wound Healing

P6. | Interactions Of Fibroblasts Versus Macrophages In An In Vitro Model Of Scar Formation And Wound Healing

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Background: After injury, a variety of different cell types are recruited and activated to facilitate healing. Excessive cell activation and recruitment leads to the overproduction and accumulation of extracellular matrix (ECM) proteins, leading to dysfunctional scar tissue. Myeloid cells, such as monocytes and macrophages, migrate to sites of injury and secrete cytokines. These cytokines recruit tissue resident fibroblasts to alter the ECM to a more fibrotic state. A better understanding of how macrophages and fibroblasts interact beyond just cytokine recruitment will help us design better therapeutics for wound healing and scar formation.

Methods: We isolated myeloid cells from the bone marrow of GFP+ mice and fibroblasts from wild type murine dermis. These cells were seeded together within a three-dimensional (3D) collagen scaffold co-culture system. These hydrogels were either left unstretched, stretched to 10% strain, or stretched to 10% strain with focal adhesion kinase (FAK) inhibition. After 2 days, the collagen cultures were explanted and the samples were processed for FACS and confocal imaging. We then repeated these 3D co-culture scaffolds with human cells, subjected to the same analysis.

Results: FACS revealed that FAK inhibited hydrogels had dramatically lower numbers of myeloid cells, while the absolute number of fibroblasts did not significantly change. FAK inhibition significantly reduced the number of CD45+ macrophages and expression of inflammatory cytokines within the hydrogels. The confocal imaging and subsequent quantification of GFP+ cells in the hydrogels also confirmed significantly lower numbers of myeloid cells. Of the myeloid cells remaining, single cell RNA sequencing showed that FAK inhibition significantly increased expression of regenerative marker EGR1 while significantly reducing the expression of fibrotic Thrombospondin proteins. Co-culturing myeloid cells with fibroblasts caused myeloid cells to differentiate so that ~8% of all cells were pro-fibrotic fibrocytes. Adding mechanical strain induced myeloid cells to become Cd86+ (classical inflammatory marker) and also increased fibrocyte differentiation to ~15%. Treatment with FAK inhibition eliminated this fibrocyte population. These findings were also observed in the human cells.

Conclusions: Blocking mechanical strain inhibits fibrotic differentiation of myeloid cells and instead shifts myeloid subpopulations towards regenerative transcriptional profiles that prevent scar formation and promote collagen architecture similar to unwounded skin. Our findings reveal a previously under-appreciated importance of myeloid cell mechanotransduction signaling during healing. This may unlock unexplored immunotherapy techniques for disease states such as fibrosis and cancer.

Directed Energy Technologies

P7. | Rose Bengal Photodynamic Therapy Improves Cutaneous Healing Of Full-Thickness Wounds In Vivo

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Introduction: Annually, over 11 million cases of significant burns require intense inpatient care; treatment costs are estimated in the trillions of dollars. Better strategies for rapid wound closure and adjuvant therapies have substantially improved survival. However, delayed wound closure and infection result in painful and debilitating scars. Novel therapies are warranted to improve wound healing. We studied the role of photodynamic compounds and organic molecule hydrogels as potential moderators of cutaneous wound healing and have identified an FDA-approved xanthene dye, Rose Bengal, as a potent inhibitor of fibrotic cell phenotype. Rose Bengal is photoactivated by monochromatic green light (MGL) at 530nm to create reactive oxygen species (ROS). In turn, the generated ROS promotes covalent bonds in collagen fibrils that produce physical crosslinks which may lead to more rapid wound closure. However, the signaling role of Rose Bengal in cutaneous wound healing in vivo has not been thoroughly explored. In this study, we report on the efficacy of photoactivated Rose Bengal therapy in a pre-clinical model of full-thickness injury.

Methods: A 6-mm punch biopsy was used to create bilateral full-thickness wounds on the dorsum of sixty-two male BALB/c mice. A silicone splint was sutured around each injury to confirm wounds healed through re-epithelialization and not contraction. Immediately after injury, animals were randomized to receive Rose Bengal (0.1% wt/vol), embedded in 10% Pluronic-F127 (PF127) hydrogel, PF127 alone, or saline alone. Treatment was applied directly to the wound bed. Animals were then exposed to MGL or placed in the dark for 30 minutes. Wound closure, total epidermal water loss (TEWL), and erythema were measured on days 3, 7, and 14. Collagen deposition and organization were measured histologically. RESULTS: Full-thickness wounds treated with photoactivated Rose Bengal had significantly more wound closure at 3 days, with full closure at day 14, compared to all other treatments ($p<0.05$). Similarly, photoactivated Rose Bengal significantly reduced TEWL (39.41 ± 5.80 g●m-2●h-1, $p<0.05$ vs. all treatments) after 7 days and showed improved wound erythema as compared to RB alone, PF127, or saline with or without MGL ($p<0.05$). Histologically, Rose Bengal photoactivation shows early wound granulation with increased angiogenesis and improved collagen organization over time compared to all other treatments.

Conclusion: These results indicate that photoactivated Rose Bengal significantly accelerates wound closure, increases collagen production, improves collagen organization, and enhances wound healing.

P8. | Improved Wound Healing And Bioburden Reduction With Novel Plasma Device

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The purpose of this study was to validate the safety and efficacy of a novel anti-infective nonthermal plasma device (Plaz4®), using an infected porcine wound model to compare bacterial bioburden and progression of wound healing in treated and untreated wounds.

Two animals received a total of sixteen (16) partial-thickness wounds, measuring 3.8 x 3.8 cm, using three passes of a dermatome. Wounds were inoculated with MRSA and left for three (3) days to develop infection. Starting Day 3, wounds were measured and assessed prior to the Plaz4 device (Plaz4) or Sham (Control) treatments. Study duration was 31 days.

No difference in wound sizes were detected between Plaz4 and Control groups prior to treatment initiation (Days 0 and 3). On Day 3, before first treatment, wounds had visible bacterial colonization and fluorescence image (MolecuLight), confirming >104 CFUs/g of bacterial colonization.

Treatments were initiated on Day 3, after wound assessments. Beginning on Day 7, after only two Plaz4 treatments (Days 3 and 5), the wound area for Plaz4 group was statistically significantly smaller than Controls. Overall, the trend of smaller wound sizes for Plaz4 persisted until the last day of the study, with difference in size statistically significant on days: 7, 18, 21 and 28. On the last day of the study there were 4 open wounds in the Control group and 1 open wound in the Plaz4 group.

Biopsies for CFU enumeration were taken from each wound on Day 21, after wounds were wiped with sterile saline and gauze to remove loosely attached bacteria. These biopsies were taken three full days after the previous Plaz4 treatment, therefore bacteria had time to proliferate between treatment and biopsy sampling. Results showed statistically significant reduction in tissue bioburden in Plaz4 treated wounds for total CFU and MRSA bacteria, 1.52 log (p=0.0047), and 1.62 log (p=0.0023) respectively. This indicates that repeated treatment with Plaz4 generated a significant bioburden reduction in wound tissues and that this effect persists for at least 3 days after treatment.

Histological analysis of the tissue samples obtained on Day 31 from Groups 1 and 2 show no differences in tissue response, supporting product safety.

This study demonstrated that Plaz4 nonthermal plasma is effective in killing MRSA bacteria in wounds, supports improved wound healing and is safe to the tissues.

P9. | Photothermal Therapy Using Gold Nanoparticles And A Long-Pulsed 755-Nm Alexandrite Laser To Treat Facial Photoaging In Asian Skin

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Background: Transdermally delivered gold nanoparticles (AuNPs) irradiated with near-infrared laser energy can create a photothermal effect within the sebaceous glands (SGs). Photothermal therapy (PTT) can be used clinically to reverse photoageing in SG-rich areas of the skin. Improvements in wrinkles and enlarged pores in Asian skin were assessed following AuNP-mediated PTT with a long-pulsed 755-nm alexandrite laser.

Methods: A single-arm, prospective trial was designed. Twenty Korean patients underwent three gold PTT interventions performed 4 weeks apart at laser hair-removal settings without cooling. At week 20, changes in the wrinkle index (WI) and pore index (PI), size, and count were calculated using 3D camera analyses. Overall improvements in wrinkles and pores (0–4 scale) were assessed by blinded investigators using standardized photographic comparisons. The subjects scored their satisfaction (0–10 scale) and treatment discomfort (0–10 scale). Safety data were also collected.

Results: There were significant reductions in the WI (8.3%, P < .01), pore size (23.1%, P = .035), and PI (19.9%, P = .034) in the periorbital areas at week 20 compared with baseline. The mean reductions in the size (22.5%, P = .027), count (16.5%, P = .048), and index (22.4%, P = .023) of the cheek pores were also significant. Investigators reported average scores of 3.2 ± 0.6 for improved wrinkle appearance, and 3.1 ± 0.7 for improvement in visible pores. Participants were moderately satisfied with the overall results (7.6 ± 1.8). Subjects felt moderate discomfort during treatment (4.7 ± 2.6). No serious or persistent adverse events occurred.

Conclusion: AuNP-mediated PTT showed noticeable efficacy and tolerability in treating wrinkles and pores in Asian skin over a 12 week-follow-up, suggesting reduction in SG activity at the parameters used. Further studies with a longer follow-up are warranted.

Dressings

P10. | The Utility of Novel Fish-Skin Derived Acellular Dermal Matrix (Kerecis) as a Wound Dressing Material

Jun Ho Park[1], Syeo Young Wee[2], Young Hoon Kim[1]

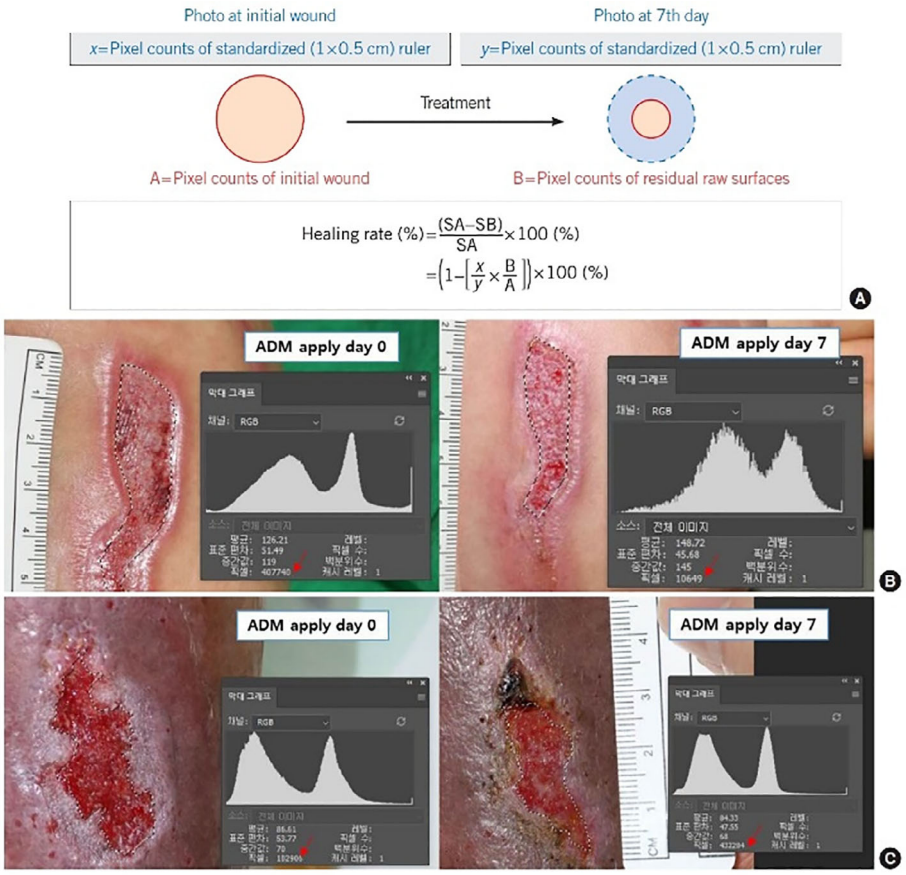
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Background: The newly-approved Kerecis is a piscine acellular dermal xenograft. This piscine acellular dermal matrix (ADM) has specific bioactive lipid mediators, omega-3 polyunsaturated fatty acids, and has a positive effect on the process of wound healing. This study aimed to explore the utility of this novel material by comparing healing rates, and suggest the proper timing for applying Kerecis.

Methods: Patients who visited the hospital with acute or chronic deep dermal wounds from June 2019 to May 2020 were enrolled in the study. A total of 48 patients were assessed. All wounds in the experimental group (n=16) were treated only once with Kerecis and a non-adherent absorptive foam material (Therasorb) to cover the ADM. In the control group, daily conventional dressings were provided. All wounds sizes were measured with mass-market computer software in a method suggested by the authors for the first time.

Results: The mean healing rate proved to be faster in the Kerecis group (P<0.05) versus the control group, and no complications were

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observed. It was statistically proved that treating burn wounds with the ADM showed better healing rates than the conventional method (P<0.05).**Conclusion:** This study establishes that managing wounds with the ADM is likely to heal wounds faster than traditional dressings. In addition, for burn wounds, a prolonged application (10 days vs. 5 days after the onset) showed a better wound healing rate (98.8% ±2.5% vs. 67.0%±14.3%, respectively, P=0.029).

P11. | Nebulized Inhalation Of A Unique Solution From A Nanocrystalline Silver Wound Dressing Has Powerful Antiviral Activity And Has Successfully Treated Patients Wth Mdr Pneumonia, Tens, And COVID-19

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Background: Antiviral and anti-inflammatory activity of a unique solution of Ag released from Acticoat was tested in standard lab and animal models. Clinical effects of nebulized inhalation of the Ag solution in ventilated patients with MDR bacterial pneumonia, TENS, and COVID-19 were assessed.

Methods: Ag release kinetics was determined using integration of absorption spectra at 350-650 nm. Inactivation of hHSV1 and SARS-CoV-2 by the Ag solution, colloidal silver, or stable silver nanoparticles was assessed using the ASTM E1052–20 protocol for antiviral testing of agents in solution. Rat lungs infected for 24 hours with Pseudomonas aeruginosa were treated with lavage of silver solution, tobramycin, or water, and rat survival and lung histology were assessed at 48 hours. Anti-inflammatory activity of the Ag solution was assessed using a pig contact dermatitis model. Eight patients, six with burn wounds who developed MDR pneumonia, one with burns who developed COVID-19, and one with TENS, all of whom were placed on ventilators, were treated with nebulized inhalation of the Ag solution and clinical parameters were measured.

Results: Silver species (Ag0, Ag+, Ag3+) were rapidly released from the nanocrystalline silver dressing into distilled water at RT, reaching 50% saturation at 24 hours and 90% saturation at 48 hours with a concentration of ~400 mg/mL. The Ag solution inactivated hHSV1 by 4-logs and 5-logs at 4 and 24 hours, and inactivated 1-log and 6-logs of SARS-CoV-2 after 4 and 24 hours, respectively. Neither a colloidal silver product nor a stable Ag nanoparticle product inactivated hHSV1 or SARS-Cov-2 viruses. Lavage of infected rat lungs with the Ag solution prevented death and preserved normal lung tissue histology. Silver nitrate or tobramycin treatments did not prevent death, and massive inflammation and lung tissue destruction was observed histologically. The Ag solution was highly anti-inflammatory in the pig contact dermatitis model. Nebulized inhalation of the Ag solution via

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ventilator rapidly reversed clinical parameters in all six patients with MDR pneumonia, in the TENS patient, and the COVID-19-infected burn patient. Chest x-rays showed no evidence of silver deposits in lung tissues of any treated patients.

Conclusions: Nebulized inhalation of a unique solution of Ag species released from the nanocrystalline silver wound dressing appears to be a highly effective new treatment for patients with MDR pneumonia, TENS, and COVID-19 due to its unique triple combination of antimicrobial, anti-inflammatory, and anti-viral actions.

P12. | Targeting Persister Hyperbiofilm Forming Bacterial Infection: The GelATA Wound Care Dressing

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Background: *Persister bacterial phenotypes such as small colony variants (SCV) are a subpopulation of antibiotic-tolerant bacterial cells that are often hyperbiofilm forming in nature. The key to managing such hostile biofilms of persister bacteria is complete eradication and one approach is to dismantle the structural framework of these biofilms. Extracellular DNA is a major component of the biofilm. DNase treatments can eradicate standard biofilms but not persister biofilms (iScience, 2019). Our work showed that, fragmented extracellular DNA (eDNA) released from a persister strain of Pseudomonas aeruginosa (PAO1ΔwspF) biofilm was responsible for resistance to disruption by DNase. PAO1ΔwspF biofilm can be disrupted by the drug aurine tricarboxylic acid (ATA), a chemical inhibitor of covalent binding between eDNA and protein. In this study, we tested the efficacy of GelATA wound care dressing (ATA incorporated into a polymer-based gel) against polymicrobial persister biofilm infection in a preclinical porcine burn wound model.*

Methods: *Eight 2"x2" full thickness burn wounds were created on the dorsum of (70-80lb) female domestic white pigs (n=4) using a standardized method published by us. Polymicrobial biofilm infection was established with clinical isolates of P.aeruginosa (PAO1 ΔwspF) and Staphylococcus aureus (S. aureus rexB). Wounds were treated with either standard of care dressing (ActicoatTM) or GelATA once weekly. At D28 postburn, treatment of GelATA treated wounds was switched to ElastogelTM alone until D56. Progression of wound healing was followed using non-invasive imaging: Digital images and Trans Epidermal Water Loss (TEWL). Histopathological examination and Scanning Electron Microscopy (SEM) of the burn wounds were performed at D56.*

Results: *SEM imaging of GelATA treated wounds showed disrupted biofilm formation with less bacterial colonization compared to ActicoatTM treatment. Furthermore, GelATA significantly ($p<0.05$) enhanced wound closure and re-epithelialization of persister biofilm-infected wounds ($p<0.05$). Interestingly, improved wound closure with inhibition of biofilm formation resulted in functional healing which was evident by significant decrease in TEWL ($p<0.05$) and improved skin barrier function in GelATA treated wounds.*

Conclusions: *This work presents first in vivo evidence for the efficacy of ATA in disrupting persister biofilm and promoting improved wound closure, functional and as photographed, in a porcine burn wound model.*

P13. | Robust Critical Limb Ischemia Porcine Model Involving Skeletal Muscle Necrosis

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Background: Critical Limb Ischemia (CLI) is the most severe and advanced stage of peripheral arterial disease (PAD), defined by impaired circulation to the lower extremities and characterized by ischemic rest pain, non-healing ischemic ulcers, and gangrene with both life and limb threatening complications. The reported similarities of the vascular and immunological systems between humans and pigs makes the porcine model relevant for pre-clinical studies. Most of the currently reported preclinical models of CLI, including porcine, are inadequate to sustain long-term ischemia causing major tissue damage. Other approaches involving upstream vascular blockade are not specific for the limb and involve pelvic ischemia causing adverse effects. This work sought to develop a surgical approach wherein pelvic blood supply remains unaffected yet the limb suffers from ischemia such that skeletal muscle necrosis is evident.

Methods: Unilateral CLI was induced by surgical transection of 1" of the common femoral artery (CFA) via infra-inguinal incision and retroperitoneal dissection in female domestic white pigs (n=5). X-ray arteriography (XRA) and computed tomography angiography (CTA) were done pre and post transection to assess the successful induction of ischemia up to d28. The effect of ischemia on limb function, perfusion, and degree of ischemic myopathy were assessed weekly by laser speckle imaging (LSI), CTA, XRA, femoral artery duplex ultrasound scans, and histologic examination.

Results: Successful induction of CLI was achieved. Gait disturbance and limping were recorded in all pigs. XRA and CTA showed complete cessation of blood flow downstream of the CFA without jeopardizing the pelvic vessels and organs. Arterial duplex indicis (pulsatility index, resistive index and systolic to diastolic ratio) were significantly lower in ischemic limb compared to contralateral limb, demonstrating successful vessel occlusion and development of ischemia ($p<0.05$). Significant decrease in skin perfusion was evident in the ischemic limb ($p<0.05$). Ischemic myopathic morphological changes with inflammatory infiltrates were evident using H&E histological analysis, associated with architectural and biomechanical changes in muscle myofibers. Such findings were corroborated with ultrasound elastography findings of increased muscle stiffness.

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Conclusions: This preclinical model robustly recapitulates features characteristic of human CLI and lends itself to mechanistic studies and therapeutic interventions.

Drug development and Translation Research

P14. | Establishment Of Ex Vivo Cultured Peripheral Blood Mononuclear Cell Therapy For Non-Healing Wounds Due To Collagen Diseases

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Background and Objective: Non-healing wounds are a major cause of morbidity and mortality in collagen disease patients. Recently, we have reported the novelty of serum free ex vivo expansion system called Quantity and Quality Culture System (QQc) using peripheral blood mononuclear cells (PbMNC) as non-invasive and effective vascular and tissue regenerative cell therapy. After demonstrating high vasculogenic and wound healing potential of this technology with murine and porcine animals, we have begun enrolling collagen disease patients with non-healing wounds in a prospective phase I/II clinical trial. The objective of this study is to investigate the safety and efficacy of MNC-QQ therapy for these patients.

Material and Methods: The first phase I study was performed by drawing 200ml of peripheral blood (PB) from collagen patients with chronic (>3 months) ischemic extremity ulcers. PbMNC were isolated and cultured in QQc for one week without passaging or media changes. Under local anesthesia, 2x107 MNC-QQc were injected within 20 cm2 of the wound. To obtain higher efficacy, we performed second stage phase I study by changing the protocol by lowering the blood draw to 100ml and injecting three times with one month interval in the calf and plantar area. The safety and efficacy were evaluated. Wound closure, VAS scale, skin perfusion pressure (SPP), TcPo2, laser doppler, thermography and angiography were performed to evaluate efficacy post therapy.

Results: Two patients of Buerger`s disease and Scleroderma were enrolled in the first stage phase I study. One patient with Malignant rheumatoid in the second stage phase I study. All the wounds extended into bone or tendon and were in the digits of the hand and foot. No major adverse effects were observed. All patients did not undergo major amputation. Significant increase in vascular perfusion with decrease in VAS scale were seen in all patients.

Conclusion: The outcomes of this prospective clinical study indicate the safety and feasibility of MNC-QQc cell therapy in patients with collagen ischemic nonhealing wounds. This methodology will allow us to transplant highly vasculogenic EPCs from small amount of blood draw. This will be the world`s first non-invasive and effective peripheral blood vascular stem cell therapy for limb salvage.

P15. | Universal Agent For Wound Infections

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Healing wounds infected with bacteria requires agents that (1) overcome drug-resistant pathogens, (2) disrupt biofilms, and (3) reduce inflammation from pathogen associated microbial molecules (PAMPS). We show data that one molecule -- 600 Da BPEI that is PEGylated to improve safety -- accomplishes these 3 goals. PEG-BPEI potentiates common antibiotics against drug-resistant Pseudomonas aeruginosa, Escherichia coli, and Klebsiella pneumoniae. These pathogens both intrinsic and acquired antibiotic resistance, making clinical management of infection a real challenge, particularly when these bacteria are sequestered in biofilms. Against Enterobacteriaceae and their biofilms, the potency of β-lactam antibiotics is restored with 600-Da branched polyethylenimine (600-Da BPEI). Checkerboard assays using microtiter plates demonstrate the potentiation of piperacillin, cefepime, meropenem, imipenem, and erythromycin antibiotics. Growth curves demonstrate that a combination of 600-Da BPEI and piperacillin produces 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 in which potentiation at low concentrations of 600-Da BPEI reduces diffusion barriers from lipopolysaccharides without disrupting the outer membrane itself. LPS also stimulates pattern recognition receptors, leading the release of pro-inflammatory cytokines. BPEI and PEG-BPEI bind to LPS to reduce cytokine production. Coupled with the ability to overcome reduce antibiotic activity and biofilms exopolymers, PEG-BPEI provides new opportunities to counter the rise of multidrug-resistant infections.

P16. | Evaluating The Antiseptic Efficacy Of On-Label Chlorhexidine Gluconate Surgical Site Preparation Kit In A Porcine Model

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Introduction: Infection in surgical wounds is dangerous and costly, significantly increasing morbidity rates and often exceeding $30,000 per case.1 Cultures from surgical site infections (SSIs) show that organisms specific to the endogenous flora of the anatomical region are the infection culprits. For example, Cutibacterium acnes is overrepresented in SSIs of the shoulders and breasts, but transdermal sites are commonly infected by Staphylococcus epidermidis and Staphylococcus aureus—standard skin flora members, suggesting that many SSIs derive from patients' skin flora.2 We hypothesized that biofilm-dwelling organisms deep in the skin survive antiseptic skin preparation

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and contribute to infection during surgical wound healing. The purpose of this study was to quantify bacterial biofilm survivors in a porcine model following an on-label chlorhexidine gluconate (CHG) presurgical scrub.

Methods: Using 4% CHG surgical skin preparation, 40 skin samples were harvested from 5 Yorkshire pigs during surgery. Control data were collected using a sterile water scrub. Pieces were immediately transferred to sterile neutralizing broth. Following tissue processing, a serial dilution was performed and plated. Plates were incubated in aerobic and anaerobic conditions, and colony forming units (CFUs) were counted and normalized per g of tissue.

Results: Aerobic and anaerobic bacteria levels following surgical scrub were 4.021x103 ± 48 and 3.748x103± 52 CFU/g, respectively. Sterile water-scrubbed tissue had 2.275x104 ± 300 and 2.149x104 ± 340 CFU/g in aerobic and anaerobic conditions, respectively.

Discussion: The data suggest that bacteria survive an alcohol-CHG scrub and remain at infectious dose levels as surgical prep reduced the total bacteria by less than one log10 unit. With the presence of a foreign body (i.e. implanted biomaterials), infectious dose was reported as low as 102 using inoculated silk sutures3. In this model, skin prep with CHG was insufficient to eliminate deep-dwelling biofilms, indicating infection risk during surgical wound healing.

References: (1) Kurts et al. J Arthroplasty (2012). (2) Williams. Targeting Biofilms in Translational Research, Device Development, and Industrial Sectors (2019). (3) Elek and Conen. British J Exp Pathol (1957).

P17. | The Use Of A Skin Morcellation Device In Skin Grafting In An Experimental Pig Acute Wound Model

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Purpose: The purpose of this study is to assess the healing of acute wounds treated with full thickness skin grafts processed by a novel skin morselizing device in an animal model

Introduction: Full thickness skin grafts, by the nature of their thickness and limited donor areas, are not optimal grafts for compromised graft beds or very large wounds. Full thickness grafts that could be morselized into thinner segments and spread out over larger areas would have the benefits of both the full thickness skin graft donor site and the expandability and thin profile of a split graft.

Methods: Full thickness wounds (2 X 2 cm) were made in the back of 3 Yorkshire Pigs in the experimental model for acute wounds in the skin graft experiment. The wounds were then treated with either morselized full thickness skin at 1:10 dilution expansion, 1:40 dilution expansion, or controls. The wounds were then treated with a 3-layer compression dressing. 2 animals were sacrificed at day 8 to assess histology and one animal was sacrificed at day 21, the end of the study. Digital images of the wound were taken at Day 0, 8, 14, and 21. Healing percentage was assessed by visual analysis with evaluation by 10 wound specialists.

Results: Results showed greatly reduced healing time in the morselized skin groups with the 1:10 dilution group showing the most rapid healing. This group showed a comparison of 10 percent versus 90 percent at 8 days for the control and 1:10 dilution respectfully. There was minimal difference between the fine and course morsels. All wounds were healed by day 21 of the study.

Conclusion: The skin grafting results were consistent with previous studies in the grafting of acute or clean wounds. Prospective human studies and wound stratification and will be needed to further access this skin grafting technology.

P18. | Cellular Tissue Product (Ctp) Use In A Hansen's Wound Case

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Background: Antimicrobial triple therapy for Mycobacterium leprae is standard of care, however, there is a paucity of information detailing the treatment of these rare wounds' care in contemporary world settings. The use of amniotic membrane stem cells and biomaterial scaffolds CTP has been used to expedite wound healing1, 2 in leprosy. This report describes the effective use of a non-human CTP matrix in a Hansen's disease case.

Methods: In April 2021, a 91 year old woman presented with right arm open ulcer. History between March 2020 and January 2021 included swelling and pain in the upper extremity, that had progressively worsened to new raised lesions and rash on trunk, and other limbs. She had no social or environmental risk factors. In March 2021, after an MRI showed an edematous skin ulcer, involving subcutaneous , biceps and brachioradialis muscles, she underwent an incisional biopsy. Diffuse non-caeseating granulomatous tissue was seen on path, with (+) AFB stains, confirmed DNA hsp65 PCR for Mycobacterium lepromatosis and negative immunostains for sarcoma, sarcoid and lymphoma.

On physical exam, the dehisced arm wound was tender with 25% necrosis, peri-wound erythema, and undermining of 2cm. X-rays were negative for osteomyelitis. Treatment consisted of repeated excisional debridement, cleansing with chlorohexidine, daily Prontosan (B.Braun Bethlehem,PA) and compression. She was referred to Bellevue hospital Hansen clinic; she was started on minocycline, and dapsone . Rifampin was added in July. Four applications of CTP (cross-linked extracellular matrix with polyhexamethylene biguanide (Pura Ply AM Organogenesis, Canton MA)) were used on the arm ulcer from July to August.

Results: Initial presentation the wound measured 5cmx6.4cmx1.2cm (32cm2). Standard of care therapy was able to reduce size to 5cmx4.9cmx0.3cm (24.5cm2) in 78 days (0.096cm2 /day). After four applications of CTP, on triple therapy and collagen with silver, the wound closed (99 days, 0.247cm2/day).

Conclusion: The adjunctive use of a cross-linked extracellular matrix with broad spectrum polyhexamethylene biguanide was an effective

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treatment modality in a patient confirmed to have Hansen's disease with skin involvement. Use of non-human CTP for leprosy may help improve healing time for open ulcers, and complement triple therapy for granulomatous disease.

1. Natallya FR et al. Effective healing of leprosy chronic plantar ulcers by application of human amniotic membrane stem cell secretome gel. Indian J Dermatol,2019 May-Jun 64(3):250

2. Sivasubramanian, S. et al. Leprosy-associated chronic wound management using biomaterials. J Glob Infect Dis. 2018 apr-jun;10 (2):99-107

P19. | Tunability Of P12, A Multi-Functional Peptide For Defined Wound Therapy

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Background: Effective wound healing requires a multitude of biologic processes. Key prerequisites include: adequate blood supply, robust granulation tissue ingrowth, increased re-epithelialization, and infection control.

Study Goal: Identify the optimal routes of administration, doses, and delivery times for P12 that address the key prerequisites for effective wound healing.

Methods: In vitro human dermal fibroblast and endothelial cell growth assays; in vivo small and large animal models to assess adequate blood supply, angiogenesis, granulation tissue formation, re-epithelialization, and rapid wound closure; and in vitro anti-bacterial assays.

Results: Cyclized P12 (cP12), at optimal 0.01mg/kg iv dose in a porcine burn model, reduces microvascular occlusion by aggregated RBCs at 24h post-burn, increases re-epithelialization at 10d post burn (Asif et al. WRR 24:501-512,2016), and promotes wound closure at 14d post-burn (data submitted to an Investigation New Drug, IND, for cP12). Pharmacokinetics of cP12 porcine studies and a cP12 Phase 1 clinical trial showed that 0.01mg/kg cP12 iv gives blood concentrations of 100pM to 10nM over the first 2 hours of administration (data submitted to an IND for cP12). Importantly, P12 at 100pM-10nM dilates the mucocutaneous microvasculature of the hamster cheek pouch (Frame et al. Microcirculation 24:e12369,2017). Thus, 100pM to 10nM cP12 blood concentrations probably clear RBC aggregates from peri-burn microvasculature in our porcine burn model via microvascular dilation and thus promote burn wound healing. In contrast, P12 at 10–30uM promotes cultured fibroblast survival and growth by biasing platelet-derived growth factor-BB (PDGF-BB) activity toward cell survival/growth and away from apoptosis in vitro (Lin et al JID134:1119-1127,2014; Zhu et al JID 134:921-929,2014), and stimulates fibroblast migration in vitro (Prasad et al WRR27:634-649,2019). Furthermore, 10–30uM P12 also stimulates in vitro and in vivo human endothelial cell angiogenesis (see McTigue et al. 2022 WHS Abstract). Importantly, cP12 applied topically at 600uM promotes early healing in acute porcine wounds (likely 10-30uM concentration deep within the wound since the delivery vehicle is <1/10 volume of wound bed)(WRR 27:634-649,2019). Recently we found that cP12 is an anti-microbial peptide (AMP) as determined by sequence analysis and by bacterial growth inhibition (100nM-1uM) and bacterial killing (10uM or higher) of P aeruginosa and S aureus. Thus, 600uM P12 applied topically at the wound surface would be a strong anti-microbial.

Conclusions: The mechanisms of action of P12 are differentially concentration-dependent and therefore tunable by changing the route of administration, dose, and/or duration of delivery. Perhaps two routes of administration, e.g. cP12 iv and topically, might be synergistic.

P20. | Evaluating The Antiseptic Efficacy Of On-Label Povidone Iodine Preoperative Skin Preparation In A Porcine Model

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Introduction: Infection is the most dangerous and costly postoperative complication, increasing morbidity rates and often exceeding $30,000 per case.1 Cultures from surgical site infections (SSIs) show that organisms specific to the flora of the anatomical region are the infection culprits.2 This data suggests that most SSIs are derived from patients' own skin flora. We hypothesize that bacteria is surviving a preoperative skin preparation of povidone iodine (PI), thus allowing bacterial survivors to contaminate surgical wound sites and contribute to infection. In this work, we quantified, biofilm-forming bacterial isolates that survived in porcine skin tissue following an on-label preoperative skin preparation with PI.

Methods: 40 porcine skin samples were harvested from five surgeries following an on-label use of PI 7.5% scrub and 10% paint surgical skin preparation kits. Control samples were collected from necropsies following a sterile water scrub for a baseline bioburden. Each sample was transferred to a sterile container with Dey-Engley neutralizing broth. Each sample was homogenized, vortexed, and sonicated. A 10-fold serial dilution was performed in duplicate. A representative count of colony forming units (CFUs) for the PI prepared skin and control skin were compared. To ensure sterility, the same procedure was performed with only Dey-Engley and plated to verify there was no contamination

Results: Aerobic and anaerobic bacteria CFUs following PI skin preparation were 1.20x103 of tissue and 7.36x102 CFU/g, respectively. Control tissue had approximately 2.27x104 and 2.15x104 CFU/g for aerobic and anaerobic, respectively.

Discussion: This data shows that PI may be insufficient to reduce biofilm burdens to below infectious dose levels. Additional work is needed to determine if bioburden levels are similar in human skin. Data indicate that if porcine and human skin respond similarly, surgeons using the very best aseptic technique and procedures may

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introduce significant levels of bacteria into surgical site wounds and contribute to post-operative infection.

References: (1) Kurts et al. J Arthroplasty (2012). (2) Williams. Targeting Biofilms in Translational Research, Device Development, and Industrial Sectors (2019).

P21. | An Unmet Need - Surgical Gloves With Variable Finger Lengths

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Purpose: Sterile gloves are essential for complex wound care and reconstructive surgery, but little research has been published on glove fit. Sterile gloves sizes 5 1/2 - 9 are produced by all manufacturers. The finger lengths are fixed for all sizes. However, many wearers have fingers that are shorter or longer than usual, causing functional limitations secondary to incorrect glove finger length and fingertip fit.

Methods: A multi question confidential electronic survey of sterile glove finger length fit was sent to 6180 glove wearers from 3 organizations; the American Society of Plastic Surgeons (ASPS), International Society of Plastic and Aesthetic Nurses (ISPAN), and the Association of Plastic Surgery Associates (APSA). Data was collected for this descriptive study in 2020 - 2021 and statistical analyses were performed using SPSS Version 2 Statistical Software.

Results: The survey yielded 500 responses, with some respondents not answering all questions. The response rate from ASPS members was 8.2%, and 7.3% from all groups combined. Most survey responders were ASPS members (86.6%); 62.7% were male. 58% had 15 or more years working in plastic surgery. The most common reason for glove finger length dissatisfaction was fingers too long (41.6%).

Of all responders, 28.51% were very likely, and 16.01%, moderately likely, to want to use gloves with longer or shorter fingers if available, a total of 44.52%. For all males total likelihood of wanting such gloves was 38.28%, and for females the likelihood of wanting gloves with variable finger lengths was 56.97%. For the z test of independent proportions there were significant differences: z = -3.86, p < .001 [95% CI = -.279, -.092]. Women were more likely to want to use short or long-fingered gloves as compared to men.

36.04% of all respondents would pay increased cost for such gloves:10% extra by 25.11% respondents, 25% extra by 11.43% respondents.

The response from all males willing to pay additional cost was 32.98% and from females 43.11%.

Conclusions: The data indicate that current sterile gloves do not support hand anatomy variations. A high percentage of sterile glove users would want gloves with variable finger lengths if such gloves were available. Better fitting sterile gloves should facilitate the processes of wound management.

Energy Metabolism in Wound Healing

P22. | Serum Metabolic Profiles Associated With Healing vs. Non-Healing In Chronic Venous Leg Ulcers

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Purpose: This study examined serum metabolic profiles associated with healing versus non-healing in chronic venous leg ulcers (CVLUs) over time in a cohort of older adults.

Methods: Serum samples (N=60) from an ongoing R01 study (R01NR016986) were collected and stored in the biorepository and then analyzed at the Southeast Center for Integrated Metabolomics (SECIM) at the University of Florida. An LC-MS untargeted metabolomics approach using high-resolution mass spectrometry (HRMS) combined with ultra-high-pressure liquid chromatography (UHPLC) was utilized to examine metabolites of 30 individuals (>50 years of age) with CVLUs at two timepoints (T1 = baseline and T2 = 4 or 8 weeks). There were 20 participants in group 1 (non-healed CVLUs) and 10 participants in group 2 (healed CVLUs). Data from positive and negative ion modes were separately subjected to statistical analyses. An ANOVA test statistic was used to examine significant changes in metabolites over time. Multivariate analysis included PCA and PLS-DA to identify significant metabolites.

Summary of Results: There were 2109 features detected from the positive mode (POS) and 2889 features in the negative mode (NEG). Identified metabolites (N =25), including Octanoylcarnitine (POS), Salicylamide (POS), Linoleic Acid (NEG), and Arachidonic Acid (NEG), significantly changed over time. The remaining (N = 230) significant metabolites are detectable; however, the chemical structure has not been identified and therefore are identified numerically. PLS-DA results revealed 54 (POS) and 42 (NEG) features were significant with a VIP score of > 2. Among the top 15 significant features in the positive and negative data sets, only 2 were known metabolites, 2-Methylglutaric Acid and Octanoylcarnitine.

Conclusion: Our data indicate LC-MS is a feasible approach for detecting significant metabolites present in serum samples of individuals with CVLUs. Significant changes in metabolites were detected, as well as significant metabolites in positive and negative data sets. Many significant metabolites are not yet identified. The utility of metabolomics in wound science is contingent upon continuing to identify and define the uncharacterized metabolites. Additional studies with larger cohorts are needed to test these concepts independently, improve understanding of healing trajectories, and develop targeted interventions for managing and treating CVLUs.

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Fibrosis and Scarring

P23. | Recurrent Bleomycin Exposure With Applied Epithelial Trauma Results In Murine Vaginal Fibrosis

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Background: Women and adolescents who undergo pelvic radiation and/or surgery often face lifelong sequelae from resulting vaginal fibrosis. Unfortunately, there are few treatment options due to the lack of vaginal fibrosis research. Our goal is to develop a reproducible vaginal fibrosis model in mice to fundamentally understand potential healing mechanisms in order to ultimately evaluate treatment and prevention options for alleviating associated symptoms for the almost 200,000 women affected.

Methods: Six to seven-week-old C57Bl/6J mice were anesthetized and instilled vaginally with normal saline (control) or 2.5U/kg bleomycin following physical trauma to induce epithelial disruption using a 0.079" diameter brush (B+ED). This was repeated 5 times over 10 days, and mice were euthanized 1 day, 3 weeks, and 6 weeks after the last instillation (n=3-7). Vaginal tissue was harvested for trichrome staining to evaluate collagen content and localization as well as flash frozen for qPCR analysis. Statistical significance was determined by t-test between control and B+ED at each time point.

Results: One day after the last instillation, there was no difference between total positive collagen staining within the vaginal submucosa between control and B+ED mice. Similarly, after 6 weeks of recovery, no difference in total collagen between control and B+ED vaginal tissue was found. However, after 3 weeks there was significantly more collagen in B+ED vaginal tissue compared to control. Additionally at 3 weeks after the last instillation, using pooled samples and a qPCR fibrosis array, we found increased Acta2, Col1a2, Col3a1, Timp3, and Timp4, as well as a decrease in Mmp13, Mmp8, and Mmp9 when comparing B+ED to control vaginal tissue.

Conclusion: Together our data suggest that epithelial disruption accompanied by bleomycin vaginal instillation results in established fibrosis at 3 weeks after the final regimen, likely through a coordinated action of increased synthesis and decreased degradation of collagen. However, the murine vagina has the ability to recover by 6 weeks. This provides us with a valuable tool to elucidate mechanisms of vaginal fibrosis and potential prevention and treatments for the many women suffering from vaginal fibrosis.

P24. | The Relationship Between Racial Identity and Scar Perception: A Comparison of Asian and Caucasian Self-Identified Patients

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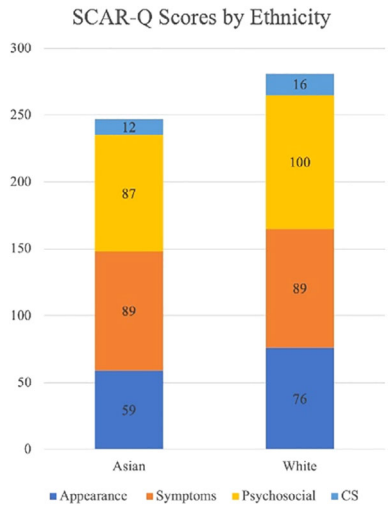
[1]Northwestern University Feinberg School of Medicine, Chicago, IL, United States [2]Texas A&M Health Science Center College of Medicine, Engineering Medicine (EnMed), Houston, TX, United States [3]Shanghai New Hongqiao Healthchoice Clinic, Shanghai,China

Background: Inappropriate scarring can result in significant morbidity that negatively impacts physical, functional, and psychosocial outcomes, particularly of the face. There remains a need to study how scarring perception varies among ethnic groups as well as scar impact on broader quality-of-life themes such as career and sexual well-being.

The objective of this study is to evaluate Caucasian and Asian patients' perception of the impact of their facial scars on symptoms, appearance, psychosocial health, career, and sexual well-being using the SCAR-Q and the CS scales.

Method: A total of 149 facial surgery patients from five providers completed the SCAR-Q, and Career/Sexual Well-Being (CS) scales via phone or email. A higher score on both assessments indicates a more positive patient perception.

Results: Of the respondents, 50.3% were Asian and 49.7% were Caucasian. Patients underwent blepharoplasty (n=56), rhinoplasty (n=21), eyelid revision (n=19), face lift (n=18), Moh's surgery (n=7), chin augmentation (n=6), craniotomy (n=6), and other procedures (n=16). Caucasian patients scored significantly better on the SCAR-Q (mean rank=86 vs. 64), appearance (mean rank=89 vs. 61), psychosocial (mean rank=83 vs. 67), and CS (mean rank=93 vs. 58) scales than Asian patients (P=.003, P<.001, P=.017, and P<.001 respectively) (Figure 1). There was a significant correlation between duration after surgery and symptoms scores for Asian (r=0.326, P=.004) and Caucasian patients (r=0.319, P=.011).**Conclusions:** Asian patients are more likely to have more negative perceptions of their scar's appearance, symptoms, psychosocial impact, career impact, and sexual well-being impact than Caucasian patients. Scar symptoms may improve over time while appearance, psychosocial, career, and sexual well-being impact may not. This study highlights the need to improve scar



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follow-up care, counseling, or other enhancement measures for patients of differing ethnicities.

P25. | Treatment of Chest Keloids Using Complete Excision Followed By A Half Keystone Flap Reconstruction Along With Immediate Postoperative [1]000cGy Radiation Therapy

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Background: Chest keloids often converged into a large lesion on the chest in some patients. Such keloids often lead to obstacles to excision and reconstruction. We describe our surgical treatment using a half keystone flap. It was subsequently followed by immediate 1000cGy radiation therapy.
Methods: We prospectively gathered patients with chest keloids between May 2021 and Dec 2021. Also, we analyzed our data from March 2017 and Dec 2020 retrospectively without radiation therapy.
Results: Of the 20 patients, Fifteen patients thought that the esthetic result was good (75%), and five patients thought the result was acceptable (25%). None patient was dissatisfied. (figure.1)
Conclusion: Half keystone flaps are effective surgical methods for managing large chest keloids. It can offer enough skin flap coverage for keloid wound resurfacing with a stable blood supply to assure satisfactory results.

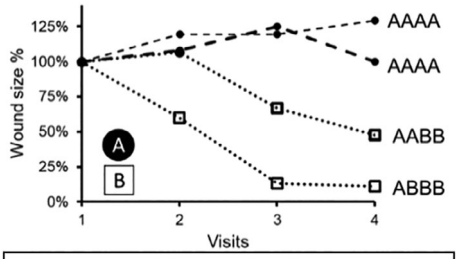
Novel Platform Technologies In Wound Healing

P26. | WITHDRAWN

P27. | Early Assessing Hyperbacric Oxygen Therapy Efficacy By Monitoring The Change Of Wound Alkalinity

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Purpose: The aim of this study was to determine whether wound alkalinity can be used as a monitoring biomarker to assess the effectiveness of hyperbaric oxygen (HBO) therapy on diabetic foot ulcers (DFUs).
Methods: Wound alkalinity was monitored by assessing discarded wound fluid-soaked wound dressings, via a disposable and non-contact device - DETEC® pH, from 16 DFU patients who have been taking continuous and indistinctive HBO treatment. The wound alkalinity at the 1st, 2nd, 3rd and 4th visits during the treatment period was correlated with the subsequent week's wound size reduction rate.
Results: The results of 16 patients with 103 alkalinities and wound size measurements were collected and used in these analyses. By plotting



Changes of wound size percentage for representative HBO treated patients. The wounds with a change of A to B were associated with a better wound size reduction compared to those remain alkaline. (AB vs AA)

line chart of wound size change over visit times (please see Figure), patients who had a wound alkalinity changing from alkaline (pH ≥ 7.0) to non-alkaline (pH < 7.0) were found with significantly larger wound size reduction than those who remained alkaline (p=0.0931 for the 3rd visit, and p=0.0266 for the 4th visit). The change of wound alkalinity (from alkaline to non-alkaline) may show the wound environment changes, perhaps the increase in inflammatory responses, which then led to increased wound size reduction. To investigate whether continuous wound alkalinity determination would help to assess HBO treatment effectiveness, the data was used to build a linear mixed effect model in which wound alkalinity was input as a fixed main effect and random effects of dependent repeated wound healing rates corresponding to the same patient and individual variations from different patients were also added. The analyses show that, under the repeated measures setup, "wound alkalinity" is a significant factor of wound healing rate (p=0.0909). The estimated coefficient corresponding to non-alkaline wound turned out to be positive (0.018499), which implies that the wound reduction rate increases when wound milieu is continuously at "non-alkaline" state.**Conclusions:** The results support that wound alkalinity may be used as a monitoring biomarker to provide quick effectiveness assessment of HBO treatment in DFUs.

Patient Research

P28. | Lower Extremity Limb Salvage In Complex Distal Third Leg And Ankle Wounds Using A Local Perforator Sparing Adipo-Fascial Turnover Flap

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Introduction: Distal third lower extremity wounds caused by trauma, arterial insufficiency, or necrotizing soft tissue infections are difficult to heal, and often result in amputations. Plastic surgeons often refer to this zone as "no man's land", in reference to the paucity of local flap options and the inability to successfully utilize free tissue transfers in the setting of comorbidities or optimized arterial insufficiency that remains incapable of supporting a free flap. We present an underutilized local flap for limb salvage in complex distal third leg and ankle wounds that would typically require amputation.

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Methods: A retrospective chart review of a single surgeon's adipo-fascial turnover flaps for lower extremity limb salvage were reviewed for demographic data, wound etiology, and outcome. Patients who had been recommended for amputation by a surgical specialist and were not a candidate for free tissue transfer reconstruction were included in the review.

Results: Seven patients aged 37-78 (mean 52) were included. The etiology of the wounds included three wounds from trauma, three from arterial insufficiency, and one from necrotizing infection. Three patients had laterally based sural perforators and four had medially based posterior tibial perforators. Donor sites were closed with autologous full thickness skin grafting. All patients had complete wound healing (2 patients required secondary operations for flap ischemia and debridement with flap advancement) without need for amputation. All patients were able to successfully ambulate postoperatively.

Conclusion: Complex distal third lower extremity and ankle wounds with exposed bone and tendons are challenging to heal by secondary intention. As a result, free tissue transfer has been the standard flap reconstruction for this anatomical region. In patients that are unable to have free tissue transfer, flap reconstruction with an adipo-fascial turnover flap should be considered prior to an amputation.

P29. | Correction of Temporal Hollowing Deformity

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Temporal hollowing deformity (THD) is a contour irregularity in the frontotemporal region, which is a common complication following surgical dissection of the temporal region or use of the coronal approach for neurosurgery. THD is characterized by a concave contour within the pterional region, along the temporal fossa and posterior to the lateral orbital rim, which results in facial asymmetry in the frontal view Here, we present our clinical experience of correction of THD using serratus anterior (SA) muscle and fascia free flaps.

Between March 2016 and December 2018, 13 patients presenting with THD were treated with SA free flap. The mean age of the patients was 47.8 years. The patients received craniectomy due to subarachnoid hemorrhage, epidural hematoma, or brain tumor. All patient underwent cranioplasty with autologous bone, poly-methylmethacrylate, or titanium mesh.

On average, the correction of THD was performed 17 months (range; 12 - 24 months) after cranioplasty. The SA muscle and fascia flap size ranged from 5 x 5 cm to 10 x 8 cm. The operation time was 107.3 minutes (range; 75 - 150 minutes). All of the flaps survived without complications. The mean follow-up duration was 20.3 months (range; 12 - 48 months). The goal of THD correction is to permanently restore aesthetic form and symmetry. Vascularized free tissue transfer provides sufficient soft tissue volume to correct THD permanently, and the robust blood supply ensures volume augmentation effect even in an irradiated or contaminated surgical field. The disadvantages of free tissue transfer include its complexity, long surgical time and hospitalization period, and donor site morbidity. For correction of THD, the SA muscle and fascia flap is among the best candidates to permanently restore aesthetic form and symmetry.



P30. | Case Study: Nutritional Supplements Support Limb Salvage Surgery For A Chronic Leg Ulcer Due To Pyoderma Gangrenosum

Mary K. Miranowski[1], Donna Dulaney[2], Efthymios Gkotsoulias[3], Deborah Thompson[2], Caroline Fife[2]

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Background: International guidelines advise use of supplemental L-arginine as part of specialized nutrition for partial or full thickness pressure injury in malnourished or at-risk adults.1 A 5-10 day protocol of high protein immunonutrition (HPIM) containing supplemental L-arginine, n-3 fatty acids and dietary nucleotides has been repeatedly demonstrated to help reduce infection and length of stay after surgery.2 Since little is known about HPIM in chronic wounds, we evaluated a 30-day course of HPIM in a malnourished adult with a full thickness chronic leg ulcer requiring limb salvage surgery.

Methods/Case Description: A 57-year-old white male had a 4-month history of a worsening right lower leg ulcer, initiated by minor trauma, but 34 cm3 in size on his first visit, with extensive exposure of tendon. Biopsy confirmed pyoderma gangrenosum; treated with high dose oral prednisone (60 mg daily), and surgery planned.

Physical exam identified muscle wasting and patient reported a 15% weight loss of usual body weight in the past month. A Mini-Nutritional Assessment3 score of 7 also indicated a malnourished patient. Clinicians stressed the importance of HPIM and after a taste test, he committed to consuming 2 HPIM cartons/day for 30 days (400 calories, 36 grams of protein and 8.4 g L-arginine daily). He was provided with a 1-month supply of HPIM samples, a record keeper, flavoring tips and a list of high protein foods. After HPIM samples were exhausted, patient utilized standard oral nutritional supplements for additional calories and protein for 3 weeks prior to surgery.

Results: Compliance with HPIM was confirmed at follow-up visits. Within one week of starting HPIM, the wound demonstrated

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increased granulation tissue, and within one month patient had gained 14 pounds. Tendon removal and successful surgical skin graft closure took place after approximately two months of nutrition intervention with healing by primary intention.

Conclusion: Despite the use of high dose steroids and pre-existing malnutrition, a highly motivated patient and health care providers committed to nutritional intervention resulted in successful limb salvage surgery including a skin graft. Clinic taste test, record keeping and removing barriers such as cost facilitated the success of specialized nutritional supplementation.

1. EPUAP, NPIAP & PPPIA. Quick Reference Guide. Emily Haesler (Ed.). EPUAP/NPIAP/PPPIA: 2019.

2. Adiamah A et al. Ann Surg 2019;270(2): 247-256.3. Kaiser MJ et al. J Nutr Health Aging 2009;13: 782-788.



P31. | Case Study: High Protein Immunonutrition Supplement Supports Limb Salvage Surgery

Mary K. Miranowski[1], Donna Dulaney[2], Efthymios Gkotsoulias[3], Deborah Thompson[2], Caroline Fife[2]

[1]Medical Affairs, Nestle Health Science, Tampa, FL, United States [2]CHI St. Luke's, The Woodlands, TX, United States [3]Greater Houston Foot and Ankle Specialists, The Woodlands, TX, United States

Background: A 5-10 day protocol of high protein immunonutrition (HPIM) containing supplemental L-arginine, n-3 fatty acids and dietary nucleotides has been repeatedly demonstrated to help reduce infection and length of stay after surgery.1 Since little is known about HPIM in chronic wounds, we evaluated a 30 -day course of HPIM in a malnourished adult with a severe ischemic foot injury requiring limb salvage surgery.

Methods/Case Description: A 34-year-old previously healthy African American female had a 21-day stay in intensive care for sepsis with hypotension and disseminated intravascular coagulopathy (DIC) causing bilateral foot ischemia. Three months later she presented to the wound center with a 15 pound weight loss, a right transmetatarsal amputation (TMA) with a non-healing area (1 cm3), and 2 ischemic ulcerations of the left plantar and lateral foot with exposed bone (70 cm3, 8 cm3). Skin perfusion pressure demonstrated that perfusion of the lateral foot was insufficient for healing (23 mmHg) and surgery was scheduled.

A Mini Nutrition Assessment2 score of 7 indicated a malnourished patient. Clinicians stressed the importance of HPIM and after a taste

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test, she committed to consuming 2 HPIM cartons/day for 30 days (400 calories, 36 grams of protein and 8.4 g L-arginine daily). She was provided with a 1-month supply of HPIM samples, a record keeper, flavoring tips and a list of high protein foods.

Results: Compliance with HPIM was confirmed at follow-up visits. Within two weeks of starting HPIM, the TMA site closed and granulation tissue was noted on left foot wounds despite tissue hypoxia. Approximately 1 week after completing HIPM, she underwent an extensive foot resection of infected bone and ischemic tissue which was closed primarily and healed without difficulty. She was ambulating 12 weeks later.

Conclusion: A highly motivated patient and health care providers committed to nutritional intervention with HPIM resulted in successful limb salvage surgery despite preoperative malnutrition and an existing non-healing surgical wound. Clinic taste test, record keeping and removing barriers such as cost facilitated the success of specialized nutritional supplementation.

1. Adiamah A et al. Ann Surg 2019;270(2): 247-256.
2. Kaiser MJ et al. J Nutr Health Aging 2009;13: 782-788.

P32. | Jet Lavage Irrigation For Severe Chronic Pelvic Pressure Injury With Modified Sodium Hypochlorite: Case Report

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We have studied daily jet lavage irrigation in the nursing home setting for the past three years with excellent safety and wound cleansing compared to the standard of care. This report discusses the addition of antiseptic as a low pressure irrigated fluid.

Methods: A 72-year-old male has a large difficult stage 4 pelvic pressure injury over the sacrum for the past 8 years that has not responded to standard of care treatment. After surgical debridement, he was entered into our investigational study where the wound was treated daily (ave. 5 days/week) with low pressure jet lavage irrigation with 3 liters of fluid (IRB #6066, Stirling Investigational Review Board, Atlanta, Ga). After 13 months treatment, we noticed that wound cleansing was not complete with our protocol. We added squeeze bottle irrigation with modified sodium hypochlorite getting the complete cleansing we sought. The IRB reviewed use of the antiseptic and approved the application through the jet lavage irrigator. We have now done 120 daily treatments over 6 months and assessed a small series of exams. After a long weekend of standard care with simple saline soak gauze dressings, we typically found a heavy bacterial growth (>log 4 colony forming units/gram of tissue) of three different bacteria, Acinetobacter baumannii, Pseudomonas aeruginosa, and group B Streptococcus. Autofluorescence imaging was done after treatment on Monday with 72 hours interval from prior treatment.

Results: After seven 'long weekends' three-day intervals, autofluorescence imaging revealed completely removed detectable bacteria by our method (Sensitivity: <log 2 colony forming units/gram of tissue). The wound appeared 'black' with the 405 NM violet light. We have identified no complications or concerns with this experience.

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Conclusions: This study confirms that jet lavage irrigation remains an important method for early wound bed preparation and bioburden debris removal. In grossly contaminated wounds, irrigation may not remove all the bacteria. Irrigating antiseptic solution under low pressure under 15 PSI has proven to be a valuable adjunct for this process.

P33. | Benefits Of Putting Into Practice The Habit Of Wholistic Patient Wound Care

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The purpose of this case report is to examine the benefits of "holistic" wound care in reducing the cost of invasive surgeries and amputations. Our patient is a 69-year-old diabetic man who presented to our wound center with a 2-month-old, painful, draining, necrotic, malodorous wound on the back of his ankle, with an exposed Achilles tendon. The patient did not know how the wound started. He was very upset and verbalized with staff about not having received appropriate care so far for his wound. He was diagnosed with mixed arterial-venous disease by a vascular surgeon.

With his medical comorbidities, our patient was at risk for severe infection and amputation. A "holistic" plan of care was established, with all wound care provided at the wound center with close monitoring. The frequency of wound center visits was 3x/week with the same team, physician visit once a week, and wound nurse twice a week. Wound care included standard care with cleaning the wound and skin with Hibiclens, topical cadexomer iodine, multilayer compression for edema control, and serial debridements. This frequency of visits was to keep the wound clean and effectively compress edema and drainage control. The patient kept the dressing and bandages on between visits. He was provided ongoing education on his condition, progress of the ulcer, and risks of wound failure. The wound has had a progressive increase in healthy granulation covering all of the Achilles tendon, new epithelization, wound contraction, and no pain. His wound is expected to heal completely. He is also more compliant and satisfied with his medical treatment and has a more positive outlook towards his recovery.

This case is small, but the magnitude of its significance is massive. It points to our ability to use cost-effective treatment protocols on wound management before it can progress to the point that requires expansive surgical procedures. Standard practice at wound centers is multiple providers for the same patient, combining wound center visits with home care, long gaps between follow-ups, resulting in wounds taking a long time to heal or having poor outcomes. What we provided is a novel concept of providing continuity and consistency of care that drives compliance, close monitoring, timely adjustments in care of the wound, ultimately reducing time to heal without costly surgical procedures.

Population Research/ Studies

P34. | A Prospective Randomized Controlled Multicenter Clinical Trial Comparing Paste-Type Acellular Dermal Matrix To Standard Care For The Treatment Of Chronic Wounds

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Background: Acellular dermal matrix (ADM) is effective for tissue regeneration and wound healing. This study was performed to compare the wound size reduction rate, epithelization rate, growth of granulation tissue, complete wound healing rate, and safety between patients receiving ADM therapy and standard wound care.

Methods: The inclusion criterion was chronic non-healing wounds, ranging from full-thickness skin defects to bone exposure, measuring > 4 cm2 that failed to heal following a minimum of 3 weeks of conservative care prior to the study. Following a 7 day screening period, patients were randomized to receive either paste-type ADM therapy or standard wound care. The wound was assessed at 0, 1, 2, 4, 8, and 12 weeks.

Results: A total of 86 patients were enrolled in the study. The wounds showed a continuous and constant reduction in size from week 1, and the wound size reduction was significantly greater compared to the control group from week 2 until the study endpoint (week 12). Granulation tissue formation was observed in 36 of 38 wounds (94.7%), and full epithelization in 34 of 38 wounds (89.5%), in the study group. Wound healing was achieved in 29 of 38 wounds (76.3%) in the study group.

Conclusions: Paste-type ADM is a useful option for wound healing and can be applied efficiently and safely for advanced wound care.

P35. | The Biobehavioral Factors Influence On Wound Healing Trajectory In Older Adults With Chronic Venous Leg Ulcers (CVLU).

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Background: Venous leg ulcers (VLUs) account for 70–90% of ulcers found in the lower leg, and they affect 2 million persons annually. VLUs have high rates of non-healing and progress to chronic wounds. The high rates of non-healing wounds are associated with multiple morbidities, local and systemic contributing factors. However, comprehensive characteristics including symptoms and molecular behaviors in the complex progression of chronic wounds in VLUs are still elusive. The purpose of this study was to characterize molecular

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behaviors from wound fluid, biological and psychological factors from patients with CVLUs during 8 weeks of wound debridement.

Methods: Participants(N=76) were recruited from a wound clinic. We collected symptoms and would fluid at baseline, week 2, 4, 6, and 8. Pain was measured by Toronto Wound Assessment. Fatigue was measured by Brief Fatigue Inventory. Wound fluid was collected by swabbing. The total activity of MMPs was measured by the change in fluorescence per unit time and reported in units of MMP-9 equivalents. Wound fluid CRP was measured by ELISA. Bayesian methods were employed to examine the strength of association among variables.

Results: Among 76 participants, 38 (54%) were male, 62 (87%) were white, and the mean age was 71.8 (SD= 10.1) years. A total of 41 patients at week 8, 32 (78%) of participants had non-healed wounds. Bayes Factor (BF) indicated that wound areas were associated with CRP (BF=11.2) and MMPs (BF=2.5). Fatigue was associated with wound areas (BF=13), CRP(BF=4) and biweekly changes of MMPs (BF=5). Pain was associated with CRP (BF=13) and biweekly changes of CRP (BF=17).

Conclusion: Evidence supports the reduction in wound area over time, and higher wound CRP is associated with larger areas of wounds. Advanced age, having comorbidities, and higher CRP and MMPs in wound fluid associated with non-healed wounds. Patients with higher wound fluid CRP tend to experience more pain. Younger patients tend to experience more pain and less fatigue. Although there is no support for wound CRP or wound MMPs moderating the change in area over time, comprehensive assessment including molecular changes from wound fluid, symptoms such as pain, and fatigue may help promote the wound healing of CVLUs.

P36. | Postoperative Flap Management using Negative Pressure Wound Therapy (NPWT) in Diabetic Foot

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Seoul National University College of Medicine, SNU-SMG Boramae Medical Center, Seoul, Korea (the Republic of)

Background: Various types of flaps are widely utilized as reconstructive options for patients with soft tissue defects. However, monitoring of the flaps still require a large amount of time and effort. The aim of this study was to evaluate the efficacy and safety of novel monitoring procedure using negative pressure wound therapy (NPWT) immediately after the flap operations especially in Diabetic Foot (DF) patients.

Methods: A retrospective analysis was performed on DF patients who underwent free flaps and perforator flaps from March 2020 to August 2021. The flaps were managed by either novel NPWT method or conventional dressing. Among NPWT group, computed tomography (CT) angiography was performed in randomly selected five flaps on the third postoperative day for evaluation of pedicle compression. Statistical analysis was performed between the two groups.

Results: A total of 26 flaps were included in this study. 14 flaps were managed using novel NPWT method and 12 flaps were managed using conventional dressing. There was no statistically significant difference in flap survival rates between the two groups (p = 0.91). The patency of flap pedicles in the NPWT group was confirmed by comparing the CT angiography findings. The estimated total flap monitoring time and cost for 5 days was significantly decreased by the application of the novel NPWT monitoring system.

Conclusion: Through the application of the novel postoperative monitoring system using NPWT, there is efficient evaluation of the flap in diabetic foot patient. Furthermore, safe flap monitoring is possible with the reduced risk of infection by the avoidance of multiple manual dressing performed in the conventional method.



Immediate application of NPWT after Free flap for Diabetic foot patient



3 months after flap coverage for diabetic foot patient

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Pressure Injuries

P37. | Effects of Polydeoxyribonucleotide in the Treatment of Pressure Injuries

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This study aimed to examine the positive effects of polydeoxyribonucleotide (PDRN) on the wound-healing process in pressure injuries. In this randomized controlled trial, the effects of PDRN were compared over time between an experimental group (n = 11) and a control group (n = 12). The former was administered the same dose of PDRN intramuscularly (1 ampule, 3 mL, 5.625 mg, for 5 days) for 2 weeks and perilesionally (1 ampule, 3 mL, 5.625 mg, twice a week) for 4 weeks. The primary endpoint for determining efficacy was wound healing in the pressure injuries, which was reflected by the wound surface area determined using VISITRAK Digital (Smith & Nephew, Largo, FL). The secondary endpoint was the pressure ulcer scale for healing score, determined using pressure ulcer scale for healing (PUSH Tool 3.0 developed by the National Pressure Ulcer Advisory Panel). After the 4-week treatment period, PDRN therapy was found to significantly reduce the wound size and PUSH score, without adverse effect during the treatment. The findings indicate that PDRN can positively modify the wound healing process in pressure injuries, and its use could improve the clinical outcomes of patients and lower the need for additional therapies or hospital stay.

Skin infection, Inflammation and Innate Immunity

P38. | Beta Adrenergic Receptor Antagonist Reduces Planktonic Growth And Biofilm Formation Of Pseudomonas Aeruginosa

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Chronic ulcers present a costly challenge to healthcare, and for patients, an increased risk of hospitalization, and poor quality of life. Though non-healing wounds may differ in etiology, a unifying feature is infection. Pseudomonas aeruginosa (PA), an opportunistic pathogen frequently identified in chronic wounds, evades the host immune system and resists antibiotic treatment by forming robust biofilms. Published studies show that the growth of some bacterial species can be modulated by catecholamines and mammalian adrenergic receptor (AR) antagonists. In this study, liquid cultures and biofilms of Pseudomonas aeruginosa strain PAO1 were treated with norepinephrine or epinephrine and timolol, a beta AR antagonist. Optical densities of liquid cultures and stained biofilms, as well as biomass and gene expression of biofilms were compared with untreated controls. In liquid culture grown for 24 hours, norepinephrine (NE) and epinephrine (EPI) increased OD of PA by 87% and 47% (n=3, 6), and timolol (TIM) reduced the OD of NE-treated cultures by 15% (n=2) ($p<0.05$). Static biofilms grown for 72 hours with NE and EPI had 2.5 and 3 times more biomass than untreated controls, and TIM reduced the biomass of EPI-treated groups by 28% (n=10, $p<0.05$). Imaging of PAO1 biofilms that were EPI-treated were found to be an average of 14% thicker than untreated controls for all timepoints, while treatment with TIM reduced thickness an average of 12% in all timepoints. Catecholamine-treated biofilms produced significantly more pyocyanin and pyoverdine than untreated biofilms (N=3, $p<0.05$). Interestingly, addition of TIM reduced gene expression for a number of genes associated with pyoverdine production, as well as others involved in biofilm formation and virulence (N=3, $p<0.05$). TIM treatment of human ex vivo skin wounded and infected with PAO1 decreased CFU/mg by two orders of magnitude ($p<0.05$). These findings demonstrate that growth of this common wound pathogen can be modulated by the catecholamines epinephrine and norepinephrine, which are present in wounds. However, the findings that these growth and biofilm effects can be partially reversed by the beta AR antagonist timolol, suggest a potential therapeutic role of timolol for chronic wounds.

P39. | Are Biofilms the Cause of Impaired Healing, or a Symptom of It?

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Background: The molecular hypotheses for wound chronicity have evolved from a lack of pro-healing growth factors, to tissue destruction by host-derived proteases, and now the bacterial biofilm hypothesis is posited to unify these hypotheses. The common idea is that wounds get stuck in a prolonged inflammatory phase. Much of current research for product development is focused on treating biofilms or reducing bioburdens in order to help the wound "turn the corner". An analysis of healing, viable biofilms, and inflammation markers in several studies has begun to cast doubt on the prolonged inflammation hypothesis; at least as it is currently formulated.
Methods: A combined post hocanalysis of clinical trial and clinical study data and literature review of non-human studies. The clinical data include trials and studies with measures of wound healing by area and volume, inflammatory markers such as matrix metalloprotease activity, local and systemic C-reactive protein, total protein, and measures of viable bacterial biofilm.
Results: The non-human studies in experimental animals are fairly clear that an injury in an health animal does not permit the formation of a biofilm. Animal models require substantial immune modulation such as induced neutropenia preceding wound inoculation, otherwise the infections are cleared prior to being able to form biofilms. In the human studies, a comparison of a given timepoint's bioburden and its levels of inflammatory markers does not reveal a strong correlation.
Conclusions: Biofilms are likely inflammatory in healthy patients, which is why otherwise healthy people do not get them. In otherwise healthy individuals with high trauma, immune saturation/depletion can provide

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a immunosuppressed window wherein biofilms can form. In the population of patients with chronic wounds, both the biofilms and lack of healing might be symptoms of an attenuated immune response.

P40. | FOXM1 signaling in diabetic foot ulcers regulates formation of neutrophil extracellular traps contributing to clinical healing outcomes

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Diabetic foot ulcers (DFU) are one of the most prevalent types of chronic wounds and are associated with a high morbidity, amputations, and mortality. DFUs are characterized by a deregulated immune response with decreased neutrophils due to loss of the transcription factor, FOXM1. Diabetes primes neutrophils to form neutrophil extracellular traps (NETs) that contribute to tissue damage and impaired healing. However, the role of FOXM1 in priming diabetic neutrophils to undergo NET formation remains unknown. We utilized a multi-disciplinary approach of genomics, murine and human models to investigate the role of FOXM1 in regulating NET formation during diabetic wound healing. Here, we found that FOXM1 regulates reactive oxygen species (ROS) levels in neutrophils and inhibition of FOXM1 resulted in increased ROS levels leading to NET formation. Next generation sequencing revealed TREM1, an amplifier of the inflammatory response highly expressed in neutrophils, as an upstream regulator of FOXM1. Activation of TREM1 reversed effects of diabetes and promoted wound healing *in vivo*. Moreover, we found that TREM1 expression correlated with clinical healing outcomes of DFUs, indicating TREM1 to be a useful biomarker and potential therapeutic target. Our data underscore TREM1/FOXM1 pathway as previously not reported regulator of NET formation during diabetic wound healing, revealing potential diagnostic and therapeutic targets that may promote healing in DFUs.

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