Abstracts

30th Annual Meeting of the Wound Healing Society SAWC-Spring/WHS Joint Meeting

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STATEMENT OF ASSURANCES:
All authors affirm that any animal studies conform with the "Position of the American Heart Association on Research Animal Use" (Circulation 1985;71:849A-850A), and that any human experimentation has been conducted according to a protocol approved by the institutional committee on ethics of human investigation or, if no such committee exists, that it conforms with the principles of the "World Medical Association Declaration of Helsinki" (Cardiovascular Research 1997;35:2-3).

WHS Abstract Session List Key

Young Investigator Awards

Concurrent Podium Presentations

Rapid Fire Poster Talks

General Poster Session

WHS Session G: Young Investigators Symposium
Thursday, April 26, 2018 1:45 P.M. - 4:00 P.M.

G.01

Wound Fluid As A Biomarker: A Metabolomic Approach

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Background- The wound fluid bathing the wound tissue reflects the wound microenvironment and shapes the functional response of wound-related cells. Building on the scientific premise that metabolites in the wound microenvironment will shape the fate of the wound, we sought to identify biochemical markers in wound fluid that can delineate between wounds that will and will not heal. Methods- Subjects (N=50) participating in the study were chronic wound patients seen at OSU Comprehensive Wound Center (CWC) clinics and have been undergoing NPWT (negative pressure wound therapy) as part of standard clinical care. Wound fluid and cells were derived from the NPWT dressing by lavaging the wound dressing with saline solution. Using different mass-spectrometry platforms, global biochemical profiles were compared in wound fluid samples from healing (>65% closure after 4 weeks) and non-healing (<20% closure after 4 weeks) wounds. Samples from each experimental group were measured and analyzed in an equivalent manner across the analytical platforms and analyzed after normalization based on measured protein values. Results- Out of 622 metabolites screened, more than a third were found to be significantly lower in the non-healing group ($p<0.05$; $n=25$) indicative of blunted tissue metabolism in wounds not engaged in active tissue repair. Consistently, the non-healing cohort exhibited decreases in metabolites linked to amino acid and polyamine homeostasis, energy utilization and lipid homeostasis ($p<0.05$; $n=25$). Interestingly, in the wound fluid of non-healing group a 3-fold increase ($p<0.05$; $n=25$) in fibrinogen-derived peptide DSGEGDFXAEGGGVR levels was noted compared to the healing cohort. This metabolite is a proteolytic fragment of fibrinogen. How this metabolite contributes to the overall proteolytic activity of the chronic wound, which is known to be high, warrants further study. Conclusion- This patient based study recognizes the value of wound fluid metabolite profile as a biomarker of wound outcome.

G.02

Mechanosensitive Lymphocytes Potentiate Wound Repair By Regulating Inflammation And Extracellular Matrix

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Background: Adaptive immune responses play a significant role in mediating tissue repair. Hyaluronan(HA), a major extracellular matrix component in skin, can influence the stiffness of the tissue and thus impact T-cell activation. However, the mechanism of action on lymphocytes is unclear. We hypothesize that lymphocytes are mechanosensitive and help govern fibrosis and wound healing. Methods: First, we tested endogenous lymphocytes' response to tension in mouse skin, and the response of human lymphocytes to hydrogels of varied stiffness. Data was analyzed by immunohistochemistry and qPCR array. Next, 6mm stented wounds were created on SCID mice, which lack functional T/B lymphocytes. Wounds were exposed to particular lymphocyte subsets by adoptive transfer of (1) total lymphocytes (TL), (2) non-CD4+ lymphocytes, or (3) CD4+ lymphocytes. Wound tissues were harvested at day7,14,30 and analyzed for wound closure(imaging), healing outcome(H&E), inflammation (CD45+ and F4/80+ cells/40X-HPF), T-cells (CD69+ cells/40X-HPF) and fibrosis(trichrome; α-SMA). Data mean±SD, p-values by ANOVA and t-test. Results: Tension increased T-lymphocyte numbers at d4 (3±0.73% vs 24.2±4.52%) in murine skin. Gene expression patterns of human lymphocytes showed dramatic changes in stiffer hydrogels, including >800-fold increase of CXCL5, and <100-fold decrease of TLR7, and 30 other inflammatory and autoimmunity genes (expression cut-off at 5-fold). Total lymphocytes, non-CD4+cells, and CD4+cells were successfully engrafted at d7 by FACS. All three lymphocyte populations reduced inflammatory infiltrates compared to SCID at d7 ($p<0.05$). TL, non-CD4+cells, and CD4+cell-treated SCID wounds showed less fibrosis compared to untreated SCID at d30 (8±2.27% vs. 10.31±3.91% vs. 13.57±3.64% vs. 21.33±6.81%). Conclusion: Our data suggests that mechanical tension strongly affects lymphocytes, and that a functional total lymphocyte population is critical to wound healing, including ECM remodeling and the attenuation of inflammation. Further characterization of defined lymphocyte subset effects will provide a better understanding of regenerative wound repair.

G.03

Caveolin 1 Inhibits Keratinocyte Migration And Wound Closure By Orchestrating Cytoskeletal Reorganization

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By oligomerization at caveolae, caveolins (cavs) can compartmentalize various signal transduction molecules, affording orchestration of transmembrane signaling events and allowing cross-talk between various downstream effectors, either sequestering certain proteins from their normal function(s) or bringing other molecules in close proximity to interact with each other. Previously, we have shown that membrane-bound glucocorticoid receptor (mbGR) mediates inhibition of keratinocyte migration during wound healing. To further understand the mechanism of this inhibition, we tested downstream targets of glucocorticoids (GC) signaling. We found that GCs upregulate cav1 expression and downregulate Rho GTPase Activating Protein 35 (ArhGAP35) expression in human keratinocytes *in vitro* and human wounds *ex vivo*. This results in increased stress fiber formation that leads to inhibition of keratinocyte migration. Furthermore, we show that upon GC treatment, cav1 co-localizes with both GR and EGFR. By disrupting caveolae, either through MβCD-mediated depletion of membrane cholesterol or CRISPR/Cas9-mediated knockdown, we detect a reversal of GR-mediated inhibition of keratinocyte migration, and a rescue of EGFR from cav1 sequestration within the caveolae, all of which improved wound healing outcomes. Interestingly, we observed that cav1 is spatiotemporally downregulated during acute wound healing and is strikingly absent from migrating epithelium, thus confirming its healing-inhibitory role. Moreover, cav1 is significantly upregulated in patients with non-healing chronic wounds, further underscoring its inhibitory role. Together our data provide evidence for a novel molecular mechanism by which cav1 inhibits keratinocyte migration and wound re-epithelialization and offers multiple novel therapeutic avenues.

G.04

ETRS Young Investigator Award Winner

Thermosensitive Biomimetic Polyisocyanopeptide Hydrogels May Facilitate Wound Repair

Roel Op 't Veld, Hans Von Den Hoff, Onno Van Den Boomen, Ditte Lundvig, Ewald Bronkhorst, Paul Kouwer, John Jansen, Frank Wagener, Alan Rowan

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Following wounding, a complex cascade of events is initiated to stop blood loss, to eliminate invading pathogens, and ultimately, to promote tissue integrity and homeostasis. However, when wound repair is interrupted severe fibrosis and scar formation can occur. Since wound dressings need to be changed regularly, this results in “ripping open” the wound area, which is painful and hampers tissue regeneration. Unfortunately, the current wound dressings are not ideal. Recently, a novel poly-isocyanopeptide (PIC) hydrogel has been developed that is thermosensitive and could facilitate in safer and painless dressing renewal. The material is liquid at cold temperatures, and gelates when temperature rises (> ~16°C). It possesses unique strain stiffening capabilities that are also similar to the natural extracellular matrix. We postulated that both this synthetic PIC hydrogel and RGD-functionalized PIC hydrogel allow normal wound repair without excessive cytotoxic or pro-inflammatory complications in full thickness wounds in the dorsal skin of mice.

We demonstrated the functionalization of PIC polymer side-chains with RGD peptides through click-chemistry. Following application of the cold liquid PIC dressings onto the wound, it immediately gelated, covering it completely. Wound closure was not hampered by the PIC hydrogels and not changed when compared to wounds without gel. No giant cells were present in wounds with or without the synthetic gel. Moreover, no inflammatory or fibrotic reactions were evoked by the biomimetic synthetic gel as we demonstrated no increase in macrophages, myofibroblasts, collagen formation, or angiogenesis. By contrast, a small but significant decrease in granulocyte recruitment was observed. Addition of RGD did not result in further changes in inflammatory cell recruitment. The observed decrease in granulocyte influx following hydrogel application is likely related to decreased bacteria influx, because the nanosized pores of the gel prevent entry. The biocompatibility and absence of adverse effects makes this tunable hydrogel highly suitable for development into a wound plaster and for targeting the different phases of woundrepair.

G.05

A Novel Sequential Multi-tiered In-vivo Approach For Quantitative Evaluation Of Topicals For Treatment Of Human Skin Scarring

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BACKGROUND Despite the abundance of topical formulations on the market for cutaneous wound and scar management, there are few with high levels of evidence. This is compounded by a relative paucity of methods for objective and quantitative evaluation of their effect in human skin. We propose a novel, multi-tiered approach for the evaluation of a topical, in particular one with an unknown mechanism of action. METHODS A randomised, blinded, clinical trial with 45 human participants compared an 'active' topical against a placebo over 16 weeks. Skin scarring was created using a 5mm biopsy punch to the upper arms and both topicals applied. Participants then received sequential biopsies of each scar. Non-invasive quantitative measurements were taken biweekly and the results validated by gene and protein studies. RESULTS This multi-tiered approach allowed for evaluation of the topical by analysing its effect on three functional parameters of skin scarring: (1) structure, (2) physiology, and (3) mechanical features. Structurally, non-invasive measurements showed an improvement in pigmentation by week 12 (W12) ($p=0.025$) by the active topical. Physiological assessment of the skin barrier showed an increase in hydration ($p<0.05$) which was validated by immunohistochemical analysis of hyaluronic acid at sequential time points, (W4 $p=0.014$, W8 $p=0.039$, and W12 $p=0.042$). Within mechanical parameters, elasticity was increased at W16 ($p=0.009$), validated by increased elastin ($p=0.044$) and fibronectin (W4 $p=0.009$, W8 $p=0.038$, and W16 $p=0.026$) levels. To detect the presence of topical within the scar tissue; Raman spectroscopy was used, which showed penetration of the topical through the scar, in the epidermis, and deep in the reticular layer. CONCLUSIONS We demonstrate for the first time, a quantitative analysis of the effect and presence of a topical in early cutaneous scar maturation over progressive sequential time points. This approach supports objective evaluation of newly emerging topicals in wound and scar management.

G.06

Mast Cell Activity During Scar Maturation: Multiple Sequential Time Point Analysis In-vivo Show Its Unique Localisation And Role In Skin Healing

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BACKGROUND Mast cells (MCs) provide mediators during wound healing and enhance acute inflammation, stimulate re-epithelialisation, angiogenesis as well as promote scarring. Their exact role in cutaneous repair remains ill-understood. Moreover, there is a paucity of evidence of their role and activity over time in human skin in vivo in cutaneous wound healing and scarring. METHODS We utilised objective noninvasive devices (full-field laser perfusion imaging (FLPI) and dynamic-optical coherence tomography (D-OCT) and detailed gene and protein studies to evaluate the time course of MC recruitment in a human wound healing model. 5mm skin-biopsies were performed on day 0 to the upper inner arms of 62 healthy volunteers, then the wounds/scars were excised at different time intervals (weeks (W)1,2,3,4,5,6,8). RESULTS MC tryptase (MCT) and chymase (MCC) analysis demonstrated that normal unwounded skin was populated with few MCs (MCT:15cells/mm2,MCC:12cells/mm2) as opposed to wounded skin and scar tissue. After reaching a maximum density at W1 compared to baseline (MCT:44cells/mm2,p=0.002; MCC:41cells/mm2,p=0.04), the MC density decreased by W8 (MCT:26cells/mm2,p=0.003; MCC:20cells/mm2,p=0.01) reaching a level similar to that observed in normal unwounded skin. Inflammation analysis by IL6 corroborated this trend with intensity values highest at W1 compared to baseline (p=0.04). VEGFA and CD31analysis confirmed the angiogenic activity of these proteases. Expression significantly increased from baseline (VEGFA:18.7%;CD31:41vessels/mm2) to W3 (VEGFA:72.8%,p=0.01; CD31:335vessels/mm2,p=0.002) and reduced to W8 (VEGFA:45.3%,p=0.02;CD31:157vessels/mm2,p=0.009). Clinical data for blood flow measured by FLPI and D-OCT supported these results demonstrating a significant increase from unwounded skin (FLPI:107.9Pu, D-OCT:0.078Au) to W1 (FLPI:394.9Pu,p<0.001; D-OCT:0.145Au,p<0.001) and reduction to W8 (FLPI:130Pu,p=0.0003; D-OCT:0.107Au,p=0.002). CONCLUSIONS For the first time in healing wounds and early scar maturation in human skin, we demonstrate the localisation of MC and its potential role in healing. These findings may help towards better understanding of the role of MC in cutaneous wound healing and scar maturation.

G.07

Single Cell Rna-seq Analyses Of Healthy Skin And Diabetic Ulcers Reveals Fundamentally Different Cell Type-specific Transcriptional Profiles

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BACKGROUND Fifteen percent of diabetes mellitus (DM) patients are expected to develop a diabetic foot ulcer (DFU) within their lifetime. DFUs represent a considerable burden for the healthcare system of both developed and developing countries. Chronic wounds, like DFUs, lack the linear progression from one classical wound healing phase to the next and are mainly characterized by the persistence of the inflammatory phase. METHODS To gain further insight on the molecular mechanisms involved in DFU development we embarked on building the transcriptomic profile of DFUs by means of single cell RNA sequencing. Discarded specimens from foot surgeries of DM patients without ulcers (n=4), DM patients with DFU following debridement process (n=2) or healthy controls (n=4) were collected and a single cell suspension was achieved with enzymatic digestion and mechanical dissociation of the tissue. Particular care was taken to ensure high cell viability. RESULTS Bioinformatics methods applied transcriptome correlation to identify distinct clusters of cells with different transcriptome states. The clusters were further annotated into specific cell types. A total of 4,142 cells were captured from the control samples, whilst 3,692 were captured from DM skin and 727 from DFUs. The most abundant cell type was as anticipated of mesenchymal origin - fibloblasts and adipocytes. Additional clusters were endothelial cells, either vascular or lymphatic, smooth muscle cells and immune cells with a clear division between T-cells and macrophages. Functions and pathways enrichment analysis of the macrophages' transcriptomic profiles revealed TREM1 and NF-kB signaling as significantly dysregulated in non-healing DFUs, while master regulator analysis highlighted STAT3 as top activated and ESR1 as top inhbited gene. CONCLUSIONS We present for the first time single cell RNA-Seq analysis of the lower extremity skin of healthy controls as well as DM patients and DFUs. Our findings provide a global view of the heterogeneity of DFU immunome and could help identify novel therapeutic targets.

G.08

Prrx1 Labels The Fibrogenic Fibroblast In The Ventral Dermis

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BACKGROUND - Scarring and fibrosis lead to excessive morbidity and mortality, as well as countless lost healthcare dollars. Despite the lack of effective therapies, there is a $12 billion annual market for the treatment of scarring in the United States alone. Herein, we identify and characterize the fibroblast sub-population responsible for scarring in the mouse ventral dermis.

METHODS - Fibroblasts with embryonic expression of Prrx1 were lineage traced by crossing $Prrx1^{Cre}$ and $ROSA26^{mTmG}$ mice. Prrx1-positive fibroblasts (PPFs) and Prrx1-negative fibroblasts (PNFs) were characterized using flow cytometry, histology, and ATAC-seq analysis at various stages of embryonic development. PPFs were ablated using triple transgenic mice expressing Cre-dependent simian diphtheria toxin receptor (DTR). Histology with picrosirius red and tensile strength testing of fully healed wounds were performed. A novel method for automated quantification of collagen fiber characteristics was performed.

RESULTS - A sub-population of fibroblasts, labeled by the embryonic expression of Prrx1, was the key contributor to connective tissue deposition during scar formation in the ventral dermis. This lineage increased as a proportion of total fibroblasts within the ventral dermis over the course of gestation, associated with the transition from scarless to scarring repair. Differential patterns of chromosomal accessibility further demonstrated the heterogeneic nature of fibroblasts. Ablation of PPFs resulted in diminished connective tissue deposition when examined after completion of wound healing ($*p<0.05$) without change in the tensile strength of the scar ($p>0.05$). PPF ablation prior to transplantation of melanoma tumor xenografts resulted in decreased tumor mass ($*p<0.05$). Analysis of collagen fiber characteristics demonstrated significant differences after PPF ablation versus control ($*p<0.05$).

CONCLUSIONS - Prrx1 identifies the fibroblast sub-population with fibrogenic potential in the ventral dermis. Selectively ablating this fibroblast sub-population leads to decreased cutaneous scarring. Further research into the role of PPFs holds promise for novel therapeutics to decrease scarring and fibrosis.

WHS SESSION H: Concurrent Session: Acute Wounds
Thursday, April 26, 2018 4:15 P.M. - 5:15 P.M.

H1.01

Inherent Features In Human Oral Epithelia Determine Heightened Wound Healing

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BACKGROUND - While oral wound healing has been considered an ideal system of wound resolution, the specific molecular events that differentiate oral wound healing are poorly understood in humans. METHODS - A human clinical study was performed to characterize the healing process and changes in gene expression between oral and cutaneous wounds. Wound healing experiments were also performed in transgenic mice. RESULTS - Oral wounds resolved faster than skin wounds in same person. RNA-sequencing, Gene Ontology, IPA and histological studies using oral and skin samples in stable state and during wound healing revealed significantly different patterns in gene expression, molecular function and biological process, especially keratinization, epidermal cell differentiation, responses to biotic stimulus, and inflammation. We identified a unique expression of the SOX2 and PITX1 transcriptional regulators that confer a specific identity on oral keratinocytes. *In vitro,* SOX2 and PITX1 had the potential of reprogramming skin keratinocytes to acquire increased cell migration capability and improve wound resolution. Lastly, skin wound healing was promoted in SOX2 overexpressing mouse (K14CreERTM/LSL-SOX2). CONCLUSIONS - We present a unique combination of human clinical data, histological and gene expression analysis, and mouse wound healing date. This information has been essential in determining the molecular anatomy of the wound healing process in oral and skin epithelia.

H1.02

Delayed Wound Healing In Usp15 Knockout Mice

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Background: Ubiquitin-specific protease-15 (USP15), which belongs to deubiquitylating enzyme family, has been found involved in transforming growth factor-beta/SMAD (TGF-β/SMAD) signaling pathway by combining SMAD7 and deubiquitination TGFβ Receptor I (TBR1). However, its role in wound repair remains unclear. Methods: In this research, USP15 knockout (USP15-KO) mice were applied and full excisional wounds were created on the dorsal skin of both USP15-KO and wild type (WT) mice. Results: General view and hematoxylin-eosin (HE) results revealed that USP15-KO mice displayed an impaired wound closure rate compared with WT mice ($p<0.05$). Immunochemistry showed that USP15-KO mice exhibited less collagen but thicker normal skin than WT mice ($p<0.05$). In addition, USP15-KO mice trended to present a low expression level of TGF-β1 and TBR1 compared with WT ($p<0.05$). Consistent with lower migration ability of USP15-KO mice epidermal cells, scratch and transwell assay *in vitro* respectively proved that Hacat cells and human dermal fibroblasts (HDF) transfected with USP15-siRNAs were significantly less motile than control. Meanwhile, G0/G1 cell cycle arrest was detected in Hacat cells after knocking down Usp15. Moreover, USP15-siRNA interfered Hacat cells presented depression of plasminogen activator inhibitor-1 (PAI-1) and TBR1 protein as well as low expression of TGF-β1 mRNA. Conclusions: These findings indicate that USP15 plays as a novel regulator both *in vivo* and *in vitro* via TGF-β1 signaling pathway and might be a potential therapeutic target in cutaneous wound healing.

H1.03

Scar Re-Pigentation: Melanocyte Repopulation In Temporal Human Skin Scarring And Its Ongoing Interaction With Inflammation And Angiogenesis

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BACKGROUND The relationship between inflammation and skin pigmentation remains ill-understood. Inflammation in scars affect re-pigmentation. The mechanisms behind this remain unclear, but immune-modulatory function of melanocytes, may provoke an inflammatory response in wound healing. METHODS The aim here was to investigate re-pigmentation of skin scarring created in 62 human subjects with similar skin-types over an 8-week period (8W) using a skin-biopsy model. Objective devices, which quantified melanin (SIAscopy), erythema (colorimeter), blood-flow (full-field laser perfusion imaging (FLPI), dynamic-optical coherence tomography (D-OCT) were used to monitor wounds/scars weekly and this was supported by detailed gene and protein analysis. RESULTS SIAscopy and colorimeter showed a significant increase from normal skin (NS) to W1 (18.8Au to 8.4Au:$p=0.004$; 30Au to 45.1Au:$p<0.001$ respectively) with a gradual reduction to W8. Masson Fontana and Melan-A analysis supported this trend (NS-W1:$p=0.04$, $p=0.05$; W1-W8:$p=0.02$, $p=0.04$ respectively) and the percentage area of epidermis in scar tissue containing melanin remained less than that of surrounding NS as pigment was absent from the centre and gradually crept in from the periphery. Erythema demonstrated the same trend ($p<0.001$). Blood flow measured by FLPI and D-OCT peaked at W1 (394.9Pu, 0.161Au respectively) ($p<0.001$) and decreased to W8 (130Pu, 0.107Au respectively) ($p<0.001$). Inflammation analysis by IL6 and IL10 intensity increased at W1 compared to NS ($p=0.04$, $p=0.03$ respectively). CD31 vessel density and VEGFA marker areas increased to W3 (105.7vessels/mm2:$p=0.002$, 72.9%:$p=0.01$ respectively) and reduced to W8. Mast-cells tryptase and chymase density increased at W1 compared to NS (44cells/mm2, $p=0.002$; 41cells/mm2,$p=0.04$ respectively), and decreased by W8 (26cells/mm2,$p=0.003$; 20cells/mm2,$p=0.01$ respectively) reaching a level similar to that observed in NS. CONCLUSIONS Here, we demonstrate, melanocyte repopulation of scars in temporal human skin biopsies and confirm the correlation between re-pigmentation, inflammation and angiogenesis. These findings may lead to better understanding and assessment of re-pigmentation in response to future scar therapies.

H1.04

Use Of Nicotine Replacement Therapy In Active Smokers Is Associated With Increased Wound Complication Rates In Breast Surgery

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PURPOSE: Previous studies have demonstrated that tobacco smoking increases the rate of surgical wound complications such as infections and delayed healing. Abstinence can help reduce these risks, but there is controversy if nicotine replacement therapy (NRT) can impact outcomes. This study aims to determine the effect of nicotine replacement therapy (NRT) on rates of healing complications of acute wounds created in patients undergoing breast surgery. *METHODS*: A retrospective chart review of female smokers undergoing breast surgery between January 2014 and April 2017 within the Yale New Haven Health System spanning across four hospitals was performed. Active smoking was defined as cigarette use within one month before or after surgery. Statistical analyses were performed using Stata software. *RESULTS*: 254 patients were identified, 34 of whom had documented NRT use six months within their breast surgery. Patient demographics such as BMI, mean age, hospital site of operation, and type of procedure performed were not significantly different between NRT-using smokers and those who did not use NRT, while race, Charlson comorbidity index, and insurance type did vary between the two groups. 52.9% of those with NRT use developed wound complications—such as infections, wound dehiscence, seromas, hematomas, tissue necrosis, fat necrosis, and lymphedema—compared to 30.5% of their non-NRT counterparts. Multivariate logistic regression accounting for covariates including age, race, BMI, Charlson comorbidity index, insurance type, race, and presence of multiple procedures resulted in a statistically significant increased risk of complication development in smokers with NRT use [OR 2.42 (1.10-5.33), p=0.027]. *CONCLUSIONS*: In our experience, concurrent NRT use in active smokers undergoing breast surgery was associated with an increased risk of postoperative wound complications compared to those not using NRT. We advise caution regarding prescribing NRT to active smokers in preparation for surgery, and recommend prospective studies to better elucidate the relationship between nicotine use and postoperative healing outcomes.

H1.05

Keratinocyte-secreted Hsp90a-containing Exosomes Are A Driving Force Of Wound Closure

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Background: Defects in tissue repair and wound healing in humans pose clinical, economic and social problems worldwide. During the entire year-long process of wound healing, only the initial period of the wound closure phase has animal models for research and has been the focus for therapeutic development. Despite decades of laboratory studies and clinical trials, few of the previous drug candidates have advanced to approval for treatment of human wounds. To overcome this deadlock, we propose that it is crucial to identify the natural "driver genes" for wound closure as new drug candidates. Methods: Acute, burn and diabetic skin wound models in mice and pigs Results: We found that when skin is injured, there is a massive deposition of heat shock protein 90alpha (Hsp90α) protein into the wound bed via keratinocyte-secreted exosomes. Although Hsp90α does not contain any membrane-anchoring motif, it is located on the surface of secreted exosomes, enabling it to bind to the LRP-1 receptor on all skin cell types to promote wound closure. Through binding to LRP-1 receptor, secreted Hsp90α exerts two critical functions to support wound closure: i) preventing cells at the wound edge undergoing hypoxia-triggered apoptosis and ii), thereafter, stimulating the cells to migrate and re-epithelialize the wound. When these two functions of keratinocyte-secreted Hsp90α were inhibited, wound closure failed to complete in a timely fashion. Finally, topical application of a recombinant Hsp90α protein that mimics keratinocyte-secreted Hsp90α, promotes wound closure in traumatic/excisional, diabetic and burn wounds in both murine and porcine models. Conclusions: A newly identified therapeutic entity of human Hsp90α, a 115-amino acid peptide, has potential as a treatment of both acute and chronic skin wounds in humans.

H1.06

Cardiac Progenitor Cell Recruitment Modulates Regulation Of Extracellular Matrix Deposition Following Myocardial Infarction

Maggie M. Hodges , Carlos Zgheib, Junwang Xu, Junyi Hu, Sarah A. Hilton, Lindel C. Dewberry, Kenneth W. Liechty

The Laboratory for Fetal and Regenerative Biology, Department of Surgery, University of Colorado School of Medicine and Anschutz Medical Campus, Aurora, CO, USA

Background: Fetal hearts possess a regenerative phenotype characterized by regeneration of functional myocardium following myocardial infarction (MI). We have demonstrated that treatment of fetal hearts with an inhibitor of stromal cell derived factor-1a (SDF1i) decreases cardiac progenitor cell (CPC) recruitment and impairs regeneration of functional myocardium. We hypothesize that inhibition of CPC recruitment after MI alters extracellular matrix (ECM) regulation, thereby promoting a fibrotic, adult phenotype in the fetal hearts treated with SDF1i.

Methods: Myocardial infarction (MI) was induced in fetal (n=18) and adult (n=5) sheep via ligation of the LAD. Quantitative PCR was used to evaluate the expression of ECM related genes (TGFB1, TGFB3, collagen 1a1, collagen 3a1, elastin, MMP-9) in adult and fetal hearts. Statistical comparisons were made using Student's t-test, with $p<0.05$ considered statistically significant.

Results: Three days following MI, fetal infarcts demonstrate attenuated expression of Col1a1, Col3a1, MMP9, Elastin, TGFB1, and TGFB3 when compared to adult infarcts ($p<0.05$). Thirty days following MI, gene expression in fetal infarcts returned to baseline, while expression of Col1a1, Col3a1, elastin, MMP-9, TGFB1, and TGFB3 remained significantly elevated in adult infarcts. Following treatment with SDF1i, Col1a1, Col3a1, MMP-9, elastin, and TGFB3 are downregulated in fetal infarcts 3 days after MI. Thirty days after MI, fetal infarcts treated with SDF1ai demonstrated upregulation of Col1a1, elastin, MMP-9, TGFB1, and TGFB3.

Conclusions: Inhibition of CPC recruitment in fetal hearts attenuates early expression of ECM related genes, while upregulating ECM related gene expression 30 days after MI, thereby conveying a fibrotic, adult phenotype to the otherwise regenerative fetal myocardium. These results are the first to suggest that CPC recruitment plays a critical role in the regulation of ECM following MI. Developing therapies targeted at increasing CPC recruitment may reduce the adverse deposition of ECM associated with the progression of heart failure.

WHS SESSION H: Concurrent Session: Infection and Biofilms
Thursday, April 26, 2018 4:15 P.M. - 5:15 P.M.

H2.01

Pf Phage In Chronic Pseudomonas Aeruginosa Wound Infections

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Background: We recently reported that a filamentous bacteriophage, Pf phage, contributes to the virulence of *Pseudomonas aeruginosa* (*P. aeruginosa*) infections in animal models but Pf contributions to human chronic wound infections are unknown. Here, we examine the clinical significance of Pf phage in patients with chronic wound infections infected with *P. aeruginosa*.
Methods: We performed a cross-sectional study of 23 patients with chronic wound infections seen at the Stanford University Advanced Wound Care Center. We collected wound swab samples and assayed these for *P. aeruginosa* and Pf phage levels. A chart review was performed to assess potential links to patient characteristics, including age, gender, diabetes status, body mass index (BMI), co-morbidities, and wound chronicity.
Results: Fifty-two samples from 23 patients were analyzed. We detected *P. aeruginosa* in 41 of 52 samples. Of these 41, 31 were positive for Pf phage, or just over 75%. On average these patients had 10^5 copies of Pf / swab (range = 10^3-10^9). Average wound chronicity for Pf positive samples was 3.26 years compared to 0.61 years in Pf negative samples, indicating a greater than five-fold increase in chronicity. Levels of Pf phage were directly correlated with *P. aeruginosa* virulence and decreased wound healing.
Conclusions: Pf phage in chronic wound infections is associated with exacerbated chronic wound infections and decreased wound healing. This data demonstrates that Pf phage may contribute to the clinical outcomes of chronic *P. aeruginosa* wound infections. This is the first report, to our knowledge, of a filamentous bacteriophage being associated with human chronic wound infections.

H2.02

Staphylococcus aureus Biofilm Infection Compromises Wound Healing By Causing Deficiencies In Granulation Tissue Collagen

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Background- *S. aureus* (SA) is one of the four most prevalent bacterial species identified in chronic wounds. Causatively linking wound pathology to biofilm properties of bacterial infection is challenging. Thus, isogenic mutant stains of SA with varying degree of biofilm formation ability, was studied in an established pre-clinical porcine model of wound biofilm infection. Methods- Pathogenic clinically isolated *S. aureus* USA300LAC (USA300) was studied. The isogenic mutant strains USA300::sar A (*ΔsarA*) and USA300::rex B (*ΔrexB*) were used as hypo- and hyper-biofilm forming mutants, respectively. The biofilm forming ability of these mutants was characterized in a preclinical persistent (8 weeks) biofilm infection model. These strains were then utilized to establish varying degree of biofilm infection in wounds as an approach to causatively link wound pathology to biofilm properties of SA infection. Results- The wound biofilm burden was significantly higher in the *ΔrexB* and USA300 in d14 and d35 burn wound biopsies, forming thick and dense aggregates of biofilm colonization on wounds compared to *ΔsarA* ($p<0.05$; n=6). Graded loss of collagen 1 expression was observed in response to varying degrees of SA biofilm infection ($p<0.05$; n=6). Significant reduction in Col1 mRNA and protein expression were noted in USA300 and Δ*rex*B infected compared to the Δ*sarA* infected wounds. Levels of the immature collagen Col3 were higher in the granulation tissue of wounds infected with Δ*rex*B. miR-143 was identified as a novel biofilm sensitive miRNA which *via* MMP-2 induction directly downregulates wound fibroblast collagen 1 levels. Conclusion- This study provides maiden evidence that chronic SA biofilm infection in wounds results in impaired granulation tissue collagen leading to compromised wound healing. Clinically, such compromise in tissue repair is likely to increase wound recidivism.

H2.03

Electrical Stimulation Signficantly Impacts Biofilm Viability, Metabolism, Biomass And Volatile Organic Compound Profiles

Mohammed Ashrafi [1], Lilyann Novak-Frazer[1], Mohamed Baguneid[2], Teresa Alonso-Rasgado[1], Riina Rautemaa-Richardson[1], Ardeshir Bayat[1]
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BACKGROUND - Antibiotic resistance and inefficiencies in monitoring response to treatment of wound infections are of significant concern. Electrical stimulation (ES) has anti-bacterial effects and could be used as an alternative to antibiotics. Microorganisms produce volatile organic compounds (VOCs) and we have previously identified specific VOC profiles of biofilms formed on human *ex-vivo* cutaneous wound models. The aim here was to assess the antimicrobial effects of ES and monitor variations in VOCs in response to ES and antibiotic therapy.

METHODS - *Staphylococcus aureus* and *Pseudomonas aeruginosa* biofilms were formed *in vitro* and subjected to either direct current ES, high dose ciprofloxacin or no treatment at early (24h) and late (72h) phases. Response to treatment was quantified using bacterial counts, metabolic (XTT) and biomass (PicoGreen) assays. Biofilm headspace was sampled at 24 hours post treatment and VOCs separated by gas chromatography and detected by mass spectrometry. VOCs were chemically identified using the National Institute of Standards and Technology library and relative abundances compared.

RESULTS - ES treated *S. aureus* biofilms showed significant reduction in bacterial counts (n=6, $P<0.001$; 24 and 72h) and biomass ($P<0.001$; 72h) compared to controls. ES treated *P. aeruginosa* biofilms showed a significant reduction in bacterial counts ($P\leq0.008$; 24 and 72h), metabolic activity ($P=0.014$; 24h) and biomass ($P=0.026$; 72h) compared to controls. There were significant variations in relative abundances of VOCs between treatment groups ($P<0.05$). Butanedione and acetic acid ethenyl ester were specific to ciprofloxacin treated *S. aureus* biofilms ($P<0.001$). *S. aureus* production of 2-methyl-1-propanol and 3-methyl-1-butanol; and *P. aeruginosa* production of hydrogen cyanide, 5-methyl-2-hexanamine, 5-methyl-2-heptanamine, 1-undecene, 3-methyl-1-butanol and 2-nonanone correlated with biofilm metabolic activity ($r\geq0.92$; $P\leq0.01$) and biomass ($r\geq0.98$; $P\leq0.0004$).

CONCLUSIONS - We conclude that ES has potential as an antimicrobial and VOC detection has prospective clinical translatability in the non-invasive monitoring of response to treatment; and human *ex-vivo* studies are currently underway.

H2.04

Polymicrobial Biofilm Infection Dysregulates Ceramide Metabolism Compromising Functional Cutaneous Wound Closure Of The Skin

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Background: Cutaneous lipids, 50% of which are ceramides (Cer), have structural and signaling roles in skin. The current study is based on our previous finding that biofilm infected wounds may appear visually closed but remain functionally open because of lack of barrier function of the repaired skin. Such defectively closed wounds display high trans-epidermal water loss (TEWL). The objective of this study was to test whether cutaneous ceramide depletion following wound biofilm infection compromises skin barrier function. Methods: Full thickness burn wounds (2"x2") were created on the dorsum of pigs and followed up to 56 days post-wounding with and without infection with mixed bacterial species consisting of *Pseudomonas aeruginosa* and *Acinetobacter baumannii,* or *Pseudomonas* ceramidase mutant strains. Analyses of wound closure (digital planimetry), skin barrier function (TEWL), ceramide levels [lipidomics, using electrospray ionization-mass spectrometry (ESI/MS), immunohistochemistry (IHC)], PPARδ, ABCA12, loricrin expression (IHC and quantitative RT-PCR), and Nile Red fluorescent staining for skin lipid distribution were performed. Results: Bacterial ceramidases were over expressed (~500 fold, n=4, $p<0.05$) in biofilm-infected pig wound tissues. Immunohistochemical studies demonstrated that biofilm-infection caused focal erosion of ceramides in the wound-edge of porcine skin. Lipidomic analyses showed that long chain ceramides were depleted in biofilm-infected porcine skin wounds. Depletion of cutaneous ceramides downregulated the transcription factor PPARδ resulting in compromised transport of lipid molecules to stratum corneum by ABCA12, a lipid transporter. Decreased PPARδ expression further lowered the expression of the keratinocyte differentiation marker, loricrin. Conclusion: Excessive microbial ceramidases in biofilm infected wounds deplete host skin ceramides. Such disruption of ceramide homeostasis in the skin causes compromised barrier function of the largest organ in the body.

H2.05

Viable Cryopreserved Umbilical Tissue (vcut) Inhibits Bacterial Growth In A Subcutaneous Rat Infection Model

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Background: Surgical site infection (SSI) and adhesions are the most common complications contributing to substantial annual morbidity, costs, and deaths. SSI is the number one reason for hospital re-admission after surgery. Efficacy of currently available adhesion barriers remains poor, and their use is associated with increased rate of SSI. *In Utero* placental tissue is a barrier that protects developing fetus including protection from infection, and therefore, placental tissue might be an ideal biological material that prevents both adhesions and SSIs. Recently, a novel viable cryopreserved umbilical tissue (vCUT) adhesion barrier/wrap/cover for surgical procedures has been developed. vCUT retains all components found in their native state. Methods: In this study we tested antimicrobial activity of vCUT in a subcutaneous rat infection model. Bilateral 3cm dorsal incisions/animal (7animals/group), and a subcutaneous pocket was created at each incision site. vCUT or collagen dressing (control) was placed in each pocket. Bacterial inoculum of *Escherichia coli*(Gr^-) or *Staphylococcus aureus*(Gr^+) were added to each pocket before skin closure. 28days post-surgery each surgical site was visually evaluated, and tissue explants collected for microbiological and histological analysis. Explants were submerged in 1ml 0.9% sterile saline and vortexed for 1 minute to elute adherent bacteria from the tissue. Serial 10-fold dilutions were plated on blood agar and incubated at 37^{o}C for 24hours before counting colony forming units (CFU). Results: Control animals (collagen dressing) developed subcutaneous abscesses at each surgical site (100%, 14/14). However, in the vCUT group the abscess was developed only at 3 sites (21%, 3/14). Degraded vCUT graft was still present at the site after 28 days as compared to the completely dissolved collagen dressing. Conclusion: vCUT inhibited bacterial growth and reduced incidence of abscess formation in an *in vivo* subcutaneous rat infection model. Data suggest that prevention of SSI might be one of the benefits provided by vCUT.

H2.06

Validation Of Biofilm Formation On Human Wound Models And Confirmation Of Their Usability In Skin-relevant Biofilm Studies

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BACKGROUND - We previously presented novel formation of common wound bacterial biofilms on human *ex-vivo* cutaneous wound models and their bacterial-specific volatile organic compound (VOC) profiles. The aims here were to further validate bacterial biofilm formation in human *ex-vivo* incisional and excisional cutaneous wound models using a further bacterial strain along with its biofilm-forming deficient derivative and biomass assay for qualitative and quantitative comparisons, respectively.

METHODS - Explant viability was assessed using XTT assay. *Staphylococcus aureus* (MSSA), *Pseudomonas aeruginosa* (PA) and *Streptococcus pyogenes* (SP) biofilms, along with biofilm-deficient *Staphylococcus aureus* mutant (SA113ΔtagO) derived from the wild type *Staphylococcus aureus* strain (SA113) were formed on plastic, incisional and excisional cutaneous wound substrates. Biofilm development was further determined at day 0, 1, 3 and 5 using histological assessment, fluorescence microscopy and biomass (PicoGreen) assay. Metabolic activity (XTT) and VOC data used in the analyses have been previously presented.

RESULTS - There were no significant differences in explant viability in *ex-vivo* models confirming their suitability over the time period ($P>0.05$). MSSA, PA and SP biofilm comparisons to SA113 and SA113ΔtagO further confirmed biofilm development and maturity. There were significant inter-strain and inter-model variations in biomass at all time points ($P<0.05$). MSSA biofilm metabolic activity correlated with biomass across all biofilm models ($R=-0.5$). PA ($R\geq0.5$) and SP ($R=-1$) biofilm metabolic activity correlated with biomass across the two cutaneous wound models. MSSA biomass correlated with 3-methyl-1-butanol ($R=-1$) across the two cutaneous wound models. PA biomass correlated with 3-methyl-1-butanol ($R=-0.5$) across the two cutaneous wound models and with hydrogen cyanide ($R\geq0.5$), 2-methyl-1-propanol ($R\leq-0.5$) and 5-methyl-2-heptanamine ($R\geq0.5$) and 2-nonanone ($R=0.5$) across all three models.

CONCLUSIONS - Further validation of biofilm formation on human cutaneous wound models confirms that these models provide a vehicle for human skin-relevant biofilm studies and VOC detection has potential clinical translatability in efficient non-invasive wound infection diagnosis.

WHS SESSION H: Concurrent Session: Bioengineering
Thursday, April 26, 2018 4:15 P.M. - 5:15 P.M.

H3.01

Photoactive Type I (atelo)collagen As Building Block Of Advanced Wound Dressings

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Diabetic patients suffer from delayed wound healing and are expected to reach more than 5 million in the UK by 2025. Advanced wound dressings have been commercialised to respond to the pressing needs of an increasing diabetic and aging population. However, control of the wound microenvironment and matrix metalloproteinases (MMPs) is still only partially accomplished, resulting in economically unaffordable healing times. Here, the use of GMP-grade type I bovine atelocollagen was explored for the design of a soluble factor- and cell-free photoactive dressing device with customisable dressing format (i.e. either wound-contacting film, photocurable liquid, or fibrous structure) and integrated wound-regulating functionalities (i.e. exudate management capability, control of chronic wound MMP activity and enhanced stability *in situ*) [1-3]. Covalent functionalisation of atelocollagen lysines with photoactive compounds, e.g. 4-vinylbenzyl chloride, was confirmed by colorimetric and spectroscopic techniques, whilst prompting the synthesis of UV-induced, water-insoluble covalent networks of preserved collagen triple helices. Atelocollagen films displayed increased water uptake (up to 2000 wt.%) and bulk compressive modulus (~80 kPa) compared to commercially available cellulose dressing products. Furthermore, MMP-9 proved to be significantly downregulated following 4-day incubation *in vitro* with atelocollagen samples, in contrast to two leading dressing products. Preclinical investigations in a full-thickness wound model in diabetic mice proved the accelerated healing capability of this soluble factor- and cell-free atelocollagen system with respect to a commercial polyurethane dressing, whilst complete wound closure was observed following 20 days post-wounding similarly to the case of cellulose dressing-treated diabetic wounds. In light of these encouraging results, a first-in-man study is currently ongoing with digital ulcers in patients with Scleroderma. [1] G. Tronci, J. Yin, R. Holmes, H. Liang, S.J. Russell, D.J. Wood. *J. Mater. Chem. B* 2016 (4) 7249[2] M.T. Arafat, G. Tronci, J. Yin, D.J. Wood, S.J. Russell. Polymer 2015 (77) 102 [3] R. Holmes, X.B. Yang, A. Dunne, L. Florea, D. Wood, G. Tronci. *Polymers* 2017 (9) 226

H3.02

Incubation Of Porcine Urinary Bladder Matrix Of Endothelial Cells And Keratinocytes From Diabetic Patients Restores A Non-diabetic Phenotype

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Background: Delayed wound healing is common in diabetic patients. Appropriate wound healing requires coordinated proliferation and migration of endothelial cells and keratinocytes. Porcine urinary bladder matrix (UBM) has been demonstrated to facilitate wound healing and decrease times to closure of diabetic foot ulcers. This study examined the impact of incubation with UBM on the phenotype of keratinocytes and endothelial cells isolated from diabetic patients. Methods: Human keratinocytes and dermal endothelial cells isolated from non-diabetic (n=3) and diabetic (n=3) donors were incubated with and without UBM powder. Total RNA was obtained from the samples and RNA-Seq analysis was performed to identify changes in RNA expression associated with exposure to UBM. Results: Principle component analysis (PCA) and hierarchical clustering demonstrated that while diabetic and non-diabetic cells initially exhibited very different RNA expression profiles, these differences were minimized following incubation with UBM. Keratinocytes incubated in UBM exhibited an increase in markers of activation, keratin 16 (2.50 ± 0.85, $p < 0.05$), keratin 6B (2.07 ± 0.79, $p < 0.05$), and keratin 6C (2.51 ± 0.90, $p < 0.05$) and down regulation of several growth factors associated with the inflammatory stage of wound healing, including Transforming Growth Factor-β1 (0.12 ± 0.04, $p < 0.05$), Connective Tissue Growth Factor (0.04 ± 0.004, $p < 0.05$), and Fibroblast Growth Factor 2 (0.09 ± 0.02, $p < 0.05$). In endothelial cells, UBM exposure was associated with decreased adhesion molecules, Intercellular Adhesion Molecule-1 (0.001 ± 0.0001, $p < 0.05$) and Platelet Endothelial Cell Adhesion Molecule-2 (0.01 ± 0.001, $p < 0.05$)and increased expression of several members of the S100A family ($p < 0.05$). Conclusion: These data suggest that exposure with UBM may restore normal cellular function in diabetic endothelial cells and keratinocytes in wounds. Larger studies are needed to evaluate more fully the effects of UBM on diabetic wound healing..

H3.03

Regeneration Of Merkel Cells In Engineered Skin Substitutes Grafted To Mice

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Background: Engineered skin substitutes (ESS) containing primary human fibroblasts and keratinocytes were shown to provide long-term closure of excised full-thickness burn wounds, but relatively little is known about innervation of ESS. Merkel cells are specialized neuroendocrine cells of the epidermis that are required for light touch sensation. The goal of this study was to begin to characterize innervation of ESS and determine if Merkel cells are present after grafting to wounds. Methods: ESS were prepared with primary fibroblasts and keratinocytes, isolated from adult human skin samples obtained with IRB approval, and were grafted to full-thickness wounds in immunodeficient mice. Biopsies were collected at multiple time points for analysis by immunohistochemistry. Results: Cells positive for Merkel cell markers keratin 18 (KRT18) and keratin 20 (KRT20) were identified in the basal epidermis of ESS by 4 weeks after grafting, suggesting the presence of Merkel cells. These cells were positive for human leukocyte antigen (HLA-ABC), confirming their human origin. Fibers staining positive for the neuronal markers NCAM and PGP9.5 were found in apposition to KRT18/KRT20-positive epidermal cells by 16 weeks after grafting, suggesting association of Merkel cells with neurons. Conclusions: The results suggest that Merkel cells were regenerated in ESS following transplantation to mice. Although we hypothesize that Merkel cells were derived from precursors present in primary keratinocyte cultures, we are currently unable to rule out the presence of rare Merkel cells in keratinocyte cultures *in vitro* prior to preparation of ESS. The results suggest that fine touch perception may be regained in healed ESS, although this must be confirmed with additional studies analyzing nerve function.

H3.04

A New Hernia Mesh Precisely Engineered to Prevent Hernia Recurrence

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PURPOSE:

Hernia repair recurrence-rate is 20% due to failure at the suture, mesh, tissue anchor-interface. We invented knitted polypropylene hernia-mesh with suture-like integrated mesh-extensions that are 15X the surface area of #0-suture and anchor the mesh akin-to-suture.

METHODS:

Polypropylene T-line mesh was fabricated with extensions 0.5-1cm-wide, 50cm-long, and spaced 2cm-apart (Table 1). Tongue-tear resistance, ball-burst, suture-retention, tensile-strength-strain, and extension-tensile-strength testing were compared to control-polypropylene-mesh. Physicomechanical-testing performed post-sterilization. Test and control-mesh were implanted in swine ventral-hernia-model and harvested 1-day post-operatively for mechanical-testing in the peri-operative period when most meshes are at greatest-risk of failure.

RESULTS:

In-vitro mechanical performance demonstrated T-line mesh outperformed control mesh in all tested parameters (Table 2). Mechanical analysis of swine-specimens demonstrated mean-peak-load to failure for T-line mesh was 134.5N(SD +/- 54.5 N), compared to 49.0N for control-reference mesh(SD +/- 13.4 N). T-line mesh failure occurred by extensions tearing through-tissue. Control-mesh failed 60% of time by one suture tearing through-tissue and second suture tearing through-mesh, remaining 40% of failures was from both sutures tearing through-mesh. T-line mesh significantly outperformed control-mesh and averaged 275% stronger on peak-load-performance.

CONCLUSION:

T-line mesh outperforms control-predicate mesh in all mechanical tests. It can be sterilized without undue effects. T-line mesh is 275%-stronger than control-mesh. Future-efforts are directed towards determining packaging-conformations, and completing tissue-toxicity testing per FDA-standards for 510(k)-clearance. T-line mesh has potential to dramatically reduce hernia-occurrence and recurrence.

H3.05

Stabilized Collagen Matrix Dressing Improves Wound Macrophage Function And Epithelialization

Mohamed S. El Masry [1], Amitava Das[1], Scott Chaffee[1], Piya Das Ghatak[1], Shomita Mathew-Steiner[1], Natalia Higuita-Castro[1], Raafat A. Anani2, Sashwati Roy[1], Chandan K. Sen[1]

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Background: Naturally derived biomaterials such as decellularized matrix of biological tissue have performed very well as wound care dressings. Such dressings present the advantage of native extracellular matrix. However, the challenge faced by any ECM-based wound care dressing product is their rapid degradation by the excessive MMPs and other proteases present in the wound environment. Stabilized, acellular, equine pericardial collagen matrix (sPCM) wound care dressing is prepared by decellularization, stabilization and sterilization of equine pericardium. However, the mechanism of action remains unclear. Methods: A murine excisional wound model was employed to study wound healing. Wound macrophages harvested from PVA sponges were used for the study. Results: The dressing was structurally characterized utilizing scanning electron and atomic force microscopy. Based on macrophage count by flow cytometric analysis and cytokine response, post-wound inflammation resolved rapidly as indicated by elevated levels of pro-resolution genes such as IL-10, Arginase-1 and VEGF and lowering of pro-inflammatory cytokines IL-1β and TNFα. sPCM induced antimicrobial proteins S100A9 and β-defensin-1 in keratinocytes ($n=5$,$^{*}p \leq 0.05$). Inhibition of biofilm formation was evident by IVIS imaging using a bioluminescent strain of *P.aeruginosa* (Xen41). Excisional wounds of C57bl/6 mice dressed with sPCM showed complete closure at day 14 while control wounds remained open ($n=10$,$^{*}p \leq 0.001$). sPCM accelerated wound re-epithelialization. sPCM not only expedited wound closure but improved the quality of healing by increased collagen deposition and maturation. Conclusion: The naturally derived biomaterial sPCM is a single-application collagen-based wound dressing capable of presenting scaffold functionality during the course of wound healing. In addition to inducing endogenous antimicrobial defense systems, it mounts robust inflammation, that rapidly resolves making way for wound healing to advance. Randomized clinical trial testing of this promising dressing material in a clinical setting is warranted.

H3.06

Implantable Oxygen Platform For Continuous, Real-time Detection Of Vascular Perfusion And Ischemia

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PURPOSE:

Lack of adequate perfusion can only be assessed post-operatively, after onset of tissue necrosis. We have developed new materials-based oxygen biosensor that can be implanted for deep and superficial measurements of tissue-oxygen-tension (TOT) in response to changes in perfusion.

METHODS:

Porphyrin-based sensors were incorporated into biocompatible hydrogels. To investigate TOT, sensors implanted intradermally and subcutaneously in rats. Sensor activity confirmed by modulating inspired oxygen levels between 12%-100%. Sensor modulation confirmed at 3,7,14 days post-implantation. Sensors similarly implanted in pigs to monitor TOT. To mimic ischemic-events, sensors directly injected at various-depths in pig-tongue which was then subject to tourniquet-ischemia.

RESULTS:

Oxygen-sensors modulated appropriately to changes in oxygen-levels. In-vivo TOT could be modulated from 0-110mmHg by modulating inspired-oxygen between 12%-100%. Sensors could also be detected in non-perfused, ex-vivo human-skin via near-infrared-fluorescence using an imaging system(IVIS). Both fluorescence and lifetime-based measurements could be obtained after at least 1cm-deep-implantations. When implanted in pigs, sensors could be monitored at 5 anatomical sites simultaneously and permitted real-time monitoring of TOT during anesthesia and euthanasia. In swine-tongue, sensors immediately detected application

WHS SESSION H: Concurrent Session: Burn Wounds
Thursday, April 26, 2018 4:15 P.M. - 5:15 P.M.

H4.01

Design And Test Of Targeted Lipid-nanoparticles In Burn Wound Care

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Background - Active-targeted lipid nanocarriers minimize off-target effects. Transferrin-conjugation strategy serves that purpose but may not be well suited for non-cancer applications such as wound. In wounds, although skin barrier function is breached, abundance of inflammatory cells at the site of injury poses a major challenge for delivery. Phagocytic clearance of nanoparticles is a threat. Methods - Novel lyophilized keratinocyte-targeted nanocarriers (TLN_{K}) were designed and loaded with anti-miR to test efficacy in treating cutaneous burn injury. TLN_{K} employed DOTAP/DODAP combination pH-responsive lipid components to improve endosomal escape. Keratinocyte-targeting was achieved using the peptide sequence ASKAIQVFLLAG. To minimize interference of clearance by non-targeted cells, especially immune cells in the acute wound microenvironment, surface charge was neutralized. Lyophilization extended shelf life of these nanoparticles. Results - Encapsulation efficiency of anti-miR in lyophilized TLN_{K} was 96.54%. Cargo stability of lyophilized TLN_{K} was tested. After 9 days of loading with anti-miR-210, TLN_{K} was effective in lowering abundance of the hypoxamiR miR-210 in keratinocytes challenged with hypoxia. Keratinocyte uptake of DiD-labelled $TLN_{K/anti\text{-}miR\text{-}107}$ was selective and exceeded 90% within 4h. Topical application of hydrogel-dispersed lyophilized $TLN_{K/anti\text{-}miR\text{-}107}$ encapsulating LNA anti-miR-107 twice a week effectively sequestered keratinocyte miR-107. On day 24, the wound area in $TLN_{K/anti\text{-}miR\text{-}107}$ treated group was reduced to 4% of the initial wound area. Barrier function of the skin, a functional measure of wound closure, as measured by trans-epidermal water loss was restored. Application of $TLN_{K/anti\text{-}miR\text{-}107}$ depleted miR-107 and upregulated dicer expression causing differentiation of keratinocytes. Expression of junctional proteins such as claudin, loricrin, filaggrin, ZO-1 and ZO-2 were significantly upregulated following $TLN_{K/anti\text{-}miR\text{-}107}$ treatment. Conclusion - The nanoparticles reported herein are promising as topical therapeutic agents in the management of burn injury. A translational advantage of TLN_{K} is that all material used for its formulation has prior history of FDA approval for human use.

H4.02

Granzyme K Impairs Wound Healing

Christopher T. Turner[1], Matthew Zeglinski[1], Hongyan Zhao[1], Phillip Bird[2], Anthony Papp[1], David Granville[1]

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Background: While once believed to be a pro-apoptotic serine protease, in recent years, the role of Granzyme K (GzmK) has been redefined due to its role in augmenting inflammation. Found at low levels in the plasma of healthy individuals, extracellular GzmK levels are markedly elevated in response to certain infections and sepsis. As burn injuries invoke a large inflammatory response, we hypothesized GzmK is increased in burn injury and contributes to prolonged inflammation and impaired wound healing. Methods: To evaluate GzmK expression, excised human burn biopsies were examined histologically. The role of GzmK was assessed in a murine model of thermal injury using wild-type or GzmK-/- mice. Wound morphometry, inflammation, Masson's Trichrome, Collagen I/III ratio and skin tensiometry were assessed. To assess a direct role for GzmK on cutaneous inflammation, cultured keratinocytes were exposed to GzmK and cell viability, cytokine expression and *in vitro* wound healing was assessed. Results: GzmK+ cells were significantly elevated in human burns compared to unwounded skin. Macrophages were the predominant cell type expressing GzmK, with M1 cells primarily responsible for both GzmK expression and secretion. A significant reduction in wound area, an increase in wound maturation and tensile strength was observed compared to equivalent burns in wild-type mice. Importantly, re-epithelialization showed the greatest degree of improvement of all parameters investigated, indicating keratinocytes may be especially susceptible to GzmK during healing. Cultured keratinocytes exposed to GzmK demonstrated impaired wound healing *in vitro* and a dose-dependent increase in pro-inflammatory cytokine release, including IL-6, which operated through a PAR-1 mediated pathway. GzmK-mediated cytotoxicity was not observed. Conclusion: GzmK contributes to impaired wound healing, potentially by prolonging the pro-inflammatory stage of wound healing.

H4.03

Reduction Of Infection And Tissue Loss In A Porcine Model Of Prolonged Field Care

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Background. In the battlefield, management of high-energy blast injuries and burns is complicated by high rates of soft tissue contamination and prolonged delays to definitive stateside care. Serious burns often result in invasive infection that can progress to septic shock and death. A critical factor in burn recovery is the time from injury to delivery of antimicrobial therapy and surgical treatment of the burn wounds. The purpose of this project was to validate the use of a hydrogel formulated with very high concentrations of antibiotics for the immediate topical care of burn wounds and determine if this treatment prevents or reduces experimentally induced infections if standard treatment is delayed for up to 7 days. Methods. Porcine partial-thickness burn wounds were created and infected with either A. baumannii, C. albicans, P. aeruginosa or S. aureus. Subsequently the burn wounds were treated topically for 7 days with a high concentration of antibiotics (1000x MIC: Minocycline, Gentamicin, Vancomycin and 10xMIC Diflucan) formulated in an alginate hydrogel. The antibiotic hydrogel treatment was compared to non-treated (blank hydrogel), silver sulfadiazine cream treated burns and to IV antibiotic treatment. On day 7 the burn wounds were harvested for quantitative bacteriology and histology. Results. The quantitative bacteriology analyses demonstrated that our antibiotic hydrogel with all the used antibiotics (n=6 wounds/group) decreased the number of bacteria in the tissue in comparison to controls. E.g 1000xMIC minocycline gel treated burns contained 1.3 log10 CFU/g and IV minocycline treated burns 6.2 log10 CFU/g bacteria ($p<0.001$). Histology showed that the hydrogel treatment reduced depth of tissue necrosis and wound area in comparison to controls. E.g depth of necrosis in 1000xMIC minocycline treated burns was 0.3 mm whereas the depth in the IV treated burns was 0.7 mm ($p<0.01$). Conclusion. It is concluded that antibiotic hydrogel stopped injury progression, minimized tissue loss and eradicated infection.

H4.04

Determination Of Adequate Debridement Of Burn Wounds Via Laser Speckle Imaging

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Background: The current standard of care for burn wound management involves removal of necrotic tissue to a bleeding wound bed then application of a skin graft depending on the depth of injury. However, the debridement procedure is difficult, time consuming, and can result in graft failure and hypertrophic scarring if performed inadequately. The purpose of this study was to determine if laser speckle imaging (LSI) could be utilized to image the debrided wound beds prior to grafting to determine if adequate debridement was performed. Methods: Deep partial and full thickness 5x5 cm burn wounds were created on the dorsum of six anesthetized Yorkshire pigs using appropriate pain control methods. After 4 days, the necrotic eschar was debrided via a dermatome to three depths (0.030", 0.060", 0.090"), a meshed split thickness skin graft was applied, and graft success (defined as >70% graft take) monitored for 14 days. LSI, which measures blood flow, was captured for all wounds pre-burn, post-burn, pre-debridement, and post-debridement. Results: Approximately 65% of wounds with the least debridement amount resulted in graft failure. LSI indicated no differences immediately post-burn but measured significant differences pre-debridement comparing burn depths. After combining all wounds into either graft success vs. failure, LSI detected higher blood flow in post-debrided wounds (0.770 vs. 0.433, respectively, $p<0.001$). Conclusions: This study confirmed the clinical situation of inadequate debridement as a contributor of graft failure. More importantly, LSI was able to differentiate between the wounds that were adequately debrided and those where necrotic tissue remained; thereby, providing clinicians with a non-invasive technique that could help determine when to graft.

H4.05

Allogeneic Cd26 / Cd55 Cell Therapy For Treating Burn Wounds

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BACKGROUND: Burns have important functional and psychosocial implications for patients. Decades of wound healing research have demonstrated a critical window within the first 24 hours after wounding during which there is a "switch" from scarless wound healing to scarring. Recently, cell-based therapies have been proposed as an option for improving healing and reducing scar formation in burn wounds. Adipose-derived stromal cells (ASCs) have become increasingly useful for cell-based therapies due to the relative ease of extraction and demonstrated performance to stimulate angiogenesis, modulate inflammation and improve wound healing.

METHODS: We have identified a stem cell population both in mice and humans with an improved wound healing profile [*Rennert et al. Nat Comm 2016*]. Single cell analysis and fluorescence-activated cell sorting were used to identify and then isolate this novel ASC subpopulation with high precision by evaluating for CD45-, CD34+, CD26+ and CD55+ cells. The murine ASC subpopulation was expanded in culture and then seeded by dual capillary seeding onto a biocompatible pullulan-collagen hydrogel. The stem cell dressing was subsequently tested on a murine contact burn model in C57Bl/6 mice to measure the regenerative capabilities of the CD26+/CD55+ ASCs.

RESULTS: Wounds treated with CD26+/CD55+ cells demonstrated accelerated healing and time to re-epithelialization, brought about by increased VEGF and SDF1 expression and significantly higher neovascularization and collagen deposition ($p<0.05$). We also observed an increase in the expression of pro-angiogenic genes MCP-1, VEGF, and SDF-1 at both the protein and mRNA level ($p<0.05$). Expression of pro-fibrotic and pro-inflammatory genes was downregulated. On average, CD26+/CD55+ ASC treated wounds closed 4 days earlier when compared to the stromal vascular fraction and ASCs. Furthermore, ASC-hydrogel treated burns exhibited reduced scar area when compared to the untreated control.

CONCLUSION: We have developed an ASC-hydrogel therapy for treating burns, with demonstrated pro-angiogenic, fibromodulatory and immunomodulatory effects. We plan to further evaluate the efficacy of CD26+/CD55+ cells in a large animal model.

H4.06

Efficacy Of Pressure Garment Therapy At Reduced Lengths Of Daily Wear

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BACKGROUND: Patient compliance is a challenge associated with pressure garment therapy. We hypothesize that if patients were able to wear the garments for less time per day, the discomforts associated with garment use would be reduced and patient compliance may improve. METHODS: To examine the effect of duration of daily wear on outcomes, full-thickness burns (1 x 1 in) were created on red Duroc pigs (8 burns/pig), excised and autografted with split-thickness skin. Adjustable pressure garments were applied 1 week after grafting and maintained at 20 $\pm$ 2 mm Hg. Garments were worn for 8, 16, or 24 hours a day for 15 weeks; control scars did not receive any pressure treatment (n=16/group). RESULTS: At 15 weeks, scars in the 24 hour/day group were approximately 50% less contracted than controls and 30% less contracted than the 8 and 16 hour/day groups ($p < 0.05$). All treatment conditions significantly reduced scar thickness vs. controls ($p < 0.05$). Scar stiffness and pliability were significantly improved over controls with just 8 hours/day of wear; however, applying pressure 24 hours a day enhanced these effects and also improved skin elasticity. CONCLUSIONS: Pressure garments worn for at least 8 hours/day are effective at reducing contraction and scar thickness and improving pliability versus controls; however, the greatest benefits in scar properties were observed with continuous use (24 hours/day).

WHS SESSION K: Concurrent Session: Chronic Wounds
Friday, April 27, 2018 2:15 P.M. - 3:15 P.M.

K1.01

Using In Vivo Label-Free Multiphoton Microscopy To Monitor Wound Metabolism

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BACKGROUND- Non-healing wounds, such as diabetic foot ulcers, are challenging to diagnose and treat due to their numerous etiologies and the variable efficacy of wound care products. There is a critical need to develop new diagnostic technologies and quantitative biomarkers that are sensitive to specific wound characteristics. Multiphoton microscopy (MPM) techniques are well-suited for 3D skin imaging and are capable of non-invasively detecting autofluorescence from metabolic cofactors (NADH and FAD) without the need for exogenous dyes. The objective of this study was to evaluate the utility of label-free MPM for characterizing wound healing *in vivo*.

METHODS- Full-thickness excisional wounds were produced in diabetic (streptozotocin-induced) and control C57BL/6J mice (n=7 mice/group). Using MPM, we isolated and measured an optical redox ratio of FAD/(NADH+FAD) autofluorescence at the wound edge to provide 3D maps of cellular metabolism over a 10-day period from each mouse.

RESULTS- A significant decrease in the optical redox ratio of the epidermis in both groups was observed between days 1 and 3 ($p<0.003$) and days 1 and 5 ($p<0.005$). By day 10, the redox ratio at the epithelial edge in the nondiabetic group had significantly increased ($p<0.03$) relative to days 3 and 5, while the diabetic mice displayed no significant temporal change. Ki-67 staining and wound closure rates indicate our optical measurements of cell metabolism are sensitive to the relative rates of keratinocyte proliferation and migration during healing. These findings demonstrate that keratinocytes at the edge of diabetic wounds remain in a proliferative state at later time points compared to control wounds.

CONCLUSIONS- Our work demonstrates label-free MPM offers potential to provide non-invasive optical biomarkers associated with different stages of skin wound healing, which may be used to detect impaired healing and guide treatment.

K1.02

Outcome Analysis Of Hyperbaric Oxygen Therapy In Diabetic Wounds And Related Gene Expression Analysis

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BACKGROUND Hyperbaric oxygen therapy (HBOT) is used to treat diabetic wounds. However, not all patients have a positive response to HBOT. We aimed to analyze the outcomes of HBOT in diabetic wounds and identify a molecular signature that differentiates HBOT-responsive from non-responsive patients to enable more cost-effective utilization of HBOT.

METHODS The medical records of patients with diabetic wounds and ≥10 HBOT treatments from September 2013 to August 2017 were reviewed. 6mm punch-biopsies were obtained from the wound beds pre-HBOT (T0) and after 7 treatments (T7). At the end of HBOT, total 7 biopsied patients were divided into responders (wound size reduction of ≥30%) and non-responders. Total RNA was extracted and RT-qPCR was performed to analyze 37 genes related to angiogenesis, inflammation, and oxidative stress. 6 housekeeping genes were selected to normalize gene expression. The threshold for changes in gene expression was set at 3-fold.

RESULTS Outcome of HBOT: 37 of 51 wounds (72.5%) were responders. Among these, 30 wounds (81.1%) completely closed. Among 14 non-responders, 6 wounds (42.9%) required amputation. Gene Expression: At T0, responders had lower relative expressions of 5 genes compared to non-responders: HSPA1A, ICAM1, TGFB1, TIMP2, and TNXB. However, at T7, these 5 genes plus TNF were up-regulated in responders compared to T0. In non-responders, CCL2 was down-regulated and TXN was up-regulated. CONCLUSIONS A 30% reduction in wound size during HBOT is a critical threshold to distinguish HBOT-responsive wounds, with significant differences in both complete wound closure and amputation rates. The responders exhibited lower gene expressions of HSPA1A, ICAM1, TGFB1, TIMP2, and TNXB before HBOT compared to non-responders, but presented notably up-regulated expression of these genes after 7 HBOT treatments; in contrast, non-responders failed to up-regulate these genes in response to HBOT. These genes could be candidates for predicting HBOT-responsiveness in future studies.

K1.03

Transdermal Deferoxamine Enhances Wound Healing In Aged Mice

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Background: There are currently 46.2 million people in the United States over 65 years old. By 2060, this number is expected to rise to 98 million. Chronic wounds, especially pressure ulcers, disproportionately affect elderly individuals causing substantial morbidity and mortality to the patient, and expense to the healthcare system. There are no effective therapies for treating chronic wounds in aged patients and most clinical trials exclude the elderly due to poorly defined outcomes and variables. We have previously shown that defective hypoxia signaling through destabilization of the master hypoxia-inducible factor 1α (HIF-1α) underlies neovascular and wound healing impairments in both aging and diabetes.

Methods: To stabilize HIF-1α, we developed a transdermal delivery system of the FDA-approved small molecule deferoxamine (DFO) and tested release of drug into the human dermis using a Franz cell set-up. For the first time we applied transdermal DFO on excisional wounds in aged mice and compared rate of healing to untreated wounds in both young and aged mice. Wound lysates were isolated and subjected to Western blotting for HIF-1α. Histological sections were stained with CD31 for new vessel formation.

Results: In vitro, transdermal DFO was consistently released into the dermis in the Franz cell setup but did not penetrate beyond the dermis. When applied on aged excisional wounds, we observed significantly accelerated wound closure ($*p<0.05$) through stabilization of HIF-1α compared to the untreated groups. There were significant improvements in neovascularization in the transdermal DFO treatment group confirmed by CD31 staining ($*p<0.05$).

Conclusions: Transdermal DFO enhances impaired wound healing in aged mice. This drug delivery system can be scaled in an FDA-compliant facility and rapidly translated to the clinic for treating both chronic wounds and pressure ulcers in aged patients.

K1.04

Epigenetic Mapping Of Wound Edge From Chronic Wound Patients Using Next Generation Sequencing

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Background- The loud biochemical microenvironment of the chronic wound sharply departs from that of the skin under homeostatic conditions and is likely to induce epigenetic changes thus influencing wound healing outcomes. Methods- Unbiased whole-genome DNA methylation (methylome) was studied in normal skin (NS) and in wound edge tissue of chronic wound (CW) patients. DNA (1 µg) isolated from CW or NS was sonicated to generate 100-300 bp size fragments followed by methylated DNA fragment enrichment and Illumina-compatible sequencing library generation. Single-end 50 bp sequencing was done using Illumina HiSeq 2500. Separate methylation status of proximal promoters (1 Kb), distal promoters (10 kb), non-CpG promoters and within exons of genes were calculated using MethylCap-Seq data analysis and PrEMeR-CG analysis. Differential methylation analysis was performed using mean vector test. Results- In proximal promoter, genes of ERK, mTOR and Notch signaling were hypomethylated. In contrast, genes involved in epithelial to mesenchymal transition were hypermethylated in CW compared to NS (p<0.0001). Hypomethylation of mTOR and ERK genes was also observed in non-CpG promoters and within exons (p<0.0001). Bisulfite sequencing was used to validate hypermethylation of candidate genes (TP53, BRCA1, and ESR1). microRNA promoters were differentially methylated in CW compared to NS (71 hyper methylated; 20 hypomethylated in CW, p<0.05). The significance of these epigenetic changes on the transcriptome was studied. A sum total of 1281 genes were found to be differentially expressed in CW compared to NS (p<0.05). Comparison between differentially methylated and differentially expressed genes identified specific genes the methylation of which downregulated expression. Examples include Potassium Voltage-Gated Channel Subfamily A Member-3, protein tyrosine phosphatase, receptor type D, microRNA-31, Glucoside Xylosyltransferase, Protocadherin-17 and Transcription factor Spi-B. Conclusion- Epigenetic activity is high at the chronic wound site. Such activity has significant impact on gene expression at the wound-edge and is therefore likely to influence healing outcomes.

K1.05

Keratinocyte-fibroblast Crosstalk Via Extracellular Vesicles Reveals Interplay Of Mirnas That Inhibits Kgf Signaling In Diabetic Foot Ulcers

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Growth factor-based therapies did not achieve expected therapeutic potential as they transitioned from pre-clinical to clinical testing. To better understand why keratinocyte growth factor (KGF/FGF7) failed to reach efficacy in clinical trial we used laser captured epidermis of wound edge tissue from diabetic foot ulcers (DFUs) and genomic profiling. We identified set of deregulated microRNAs (miRs) including miR-31-5p and miR-15-5b among the top induced. One of predicted targets of miR-31-5p is KGF/FGF7, a stimulator of keratinocyte migration produced by fibroblasts. Consistent with epidermal miR-31-5p induction, we found suppression of FGF7 in DFU fibroblasts and validated FGF7 as miR31-5p target by 3' UTR luciferase reporter assays. Furthermore, overexpression of miR-31-5p into the target cells, primary fibroblasts, caused suppression of FGF7, even in the presence of major stimulator, IL1β. Thus, we postulated that sustained miR-31-5p overexpression by keratinocytes in DFUs targets FGF7 in fibroblasts in a paracrine manner. Indeed, we found miR-31-5p to be secreted in extracellular vesicles (EVs) from keratinocytes, verified by nanoparticle tracking analysis. EVs containing miR-31-5p were functionally active in suppressing FGF7 in fibroblasts and in organotypic human skin. KGF/FGF7 signaling targets keratinocytes via FGFR2. We also found down-regulation of FGFR2 in DFU epidermis. miR-15b-5p and miR-424-5p, over-expressed in epidermis of DFUs, both target FGFR2. Their induction and direct targeting of FGFR2 was verified in DFU tissue and by luciferase reporter assays, respectively. We conclude that KGF/FGF7 signaling is impaired in DFUs via complex mechanism that involves over-expression of miR-31 in keratinocytes that targets and suppresses FGF7 in fibroblasts via EVs. Furthermore, suppression of FGFR2 in keratinocytes via miR-15b-5p and miR-424-5p leads to complete impairment of KGF-mediated effects, which provides insights into molecular mechanisms that precluded successful outcomes of recombinant KGF therapies.

K1.06

Transdermal Deferoxamine Significantly Enhances Healing Of Sickle Cell Ulcers

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Background: Sickle cell disease (SCD) affects approximately 100,000 people in the United States and is associated with hemochromatosis. Roughly 2.5% of this patient population experience sickle cell ulcers (SCU), with the incidence increasing to 27% in patients in low income areas. SCUs form over the medial or lateral malleoli and are prone to infection and recidivism. Some ulcers can last up to 20 years or may never heal leading to pain, clubbed feet or amputations. There is no effective therapy for treating SCUs.

Methods: Deferoxamine is FDA-approved for the treatment of hemochromatosis and we have previously found deferoxamine to heal diabetic ulcers in murine models. Thus, we developed a clinical-grade transdermal deferoxamine delivery system (TDDS) for treating SCUs. TDDS was applied daily on excisional wounds in a murine model of SCD and compared to untreated wounds or wounds subcutaneously injected with deferoxamine. High performance liquid chromatography (HPLC) was used to test DFO release from the delivery system 24 hours following application on the wound. 50um wound sections were subjected to inductively coupled mass spectrometry to test for free iron.

Results: TDDS-treated wounds demonstrated significantly accelerated time to closure ($p<0.05$), reduction in wound size and improved wound remodeling as demonstrated by increased collagen deposition. HPLC confirmed release of deferoxamine from the TDDS into the dermis of both wounded skin and re-epithelialized skin. Plasma mass spectrometry demonstrated that TDDS chelated excessive free iron within the dermis as there was significantly lesser Fe^{2+} and Fe^{3+} ($p<0.05$) in this treatment group.

Conclusions: TDDS significantly enhances healing of ulcers by chelating excessive free-iron in a murine model of sickle cell disease. TDDS is currently being manufactured in an FDA-compliant and ISO-13485 facility to be rapidly translated for treating patients with sickle cell ulcers.

WHS SESSION K: Concurrent Session: Chronic Wounds & Inflammation
Friday, April 27, 2018 2:15 P.M. - 3:15 P.M.

K2.01

A Modified Collagen Gel Resolves Wound Inflammation Via Microrna-21-dependent Pro-healing Macrophage Polarization

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Background- Collagen based dressings are widely used in wound care yet their mechanisms of action remain to be understood. Previous studies using a modified collagen gel (MCG) dressing demonstrated robust vascularization of ischemic wounds and improved healing outcomes. Wound macrophages play a critical role in enabling wound angiogenesis and timely healing. Thus, in this work, we sought to investigate the direct action of MCG dressing on wound macrophage phenotype and function. Methods- Wound cells were isolated from MCG treated PVA sponges implanted subcutaneously on the back of mice. Results- MCG increased macrophage recruitment to the wound site and promoted polarization to pro-healing ($m\phi^{heal}$) phenotype indicative of robust inflammation followed by timely resolution ($p<0.05$; $n=4$). Increased $m\phi^{heal}$ phenotype polarization was associated with copious production of anti-inflammatory cytokine IL-10 and proangiogenic VEGF suggesting a direct action of MCG on wound macrophages in supporting resolution of inflammation and improving angiogenesis ($p<0.05$; $n=4$). Impaired clearance of apoptotic cell bioburden at wound-site feeds chronic inflammation. Previous studies in our laboratory reported that engulfment of apoptotic cells by macrophages (efferocytosis) drives polarization of pro-inflammatory macrophages ($m\phi^{inf}$) to $m\phi^{heal}$ *via* a miR-21-PDCD4-IL-10 pathway. Significantly increased ($p<0.05$; $n=4$) efferocytosis index was noted in macrophage from MCG treated wounds. Such favorable outcome resulted in a significant induction ($p<0.05$; $n=4$) of miR-21 expression. Implicating miR-21 as a causative factor, MCG mediated induction of IL-10 in wound macrophages was blunted under conditions of miR-21 knockdown by miR-21-zip. Pharmacological inhibition of JNK in macrophages resulted in attenuated IL-10 production by MCG, indicating a role of miR-21-JNK pathway in MCG-mediated IL-10 release in macrophages. Conclusion- The findings of this work provide a novel paradigm in macrophage-ECM interactions as well as reshape the understanding of the mechanisms of action of collagen based dressings in the treatment of chronic wounds.

K2.02

Physiological Cell Reprogramming At The Site Of Tissue Injury Critical Role Of Mir-21

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Background-Wound healing is a complex and dynamic process of repairing and restoring injured tissues. We propose that the wound mileu acts as a cradle for physiological cell reprogramming. Such reprogramming plays a critical role in tissue repair. MicroRNAs (miRs) are small non-coding RNAs that enact post-transcriptional gene silencing. miRs determine the pattern of the injury-inducible transcriptome and therefore healing outcomes. In 2009, our laboratory reported a critical role of miR-21 in myocardial injury, cited>600x. Methods- A tamoxifen inducible $K14^{Cre}miR\text{-}21^{\Delta/\Delta}$ mice model was developed resulting in targeted knock down of miR-21 is epithelial of skin. Results-Resolution of inflammatory phase was delayed in wounds of K-14Cre-miR-$21^{-/-}$ mice. Immuno-histochemical staining revealed abundance of macrophages at day 10 post-wounding ($p<0.05$). Cytokine analyses from the wound fluid of these transgenic mice revealed increased abundance of inflammatory cytokines regulated by miR-21. $K14^{Cre}miR\text{-}21^{\Delta/\Delta}$ mice showed compromised quality of healed wounds as demonstrated by reduced collagen (Masson's trichrome staining; $p<0.05$). Transition of one cell type to the other is emerging to be a very vital process in tissue remodeling. We observed transition of macrophages to fibroblast-like cells at the site of injury and found that this transition was blunted in $K14^{Cre}miR\text{-}21^{\Delta/\Delta}$ mice. Through *in vitro* and *in vivo* experiments, we found that miR-21 played a crucial role in shifting the equilibrium towards conversion of macrophages to fibroblast-like cells. miR-21 levels are reduced in pathological conditions, such as diabetes which compromises conversion of macrophages to fibroblast-like cells, thus resulting in impaired wound healing. Conclusion-Conversion of one cell type to another is a part of the normal physiologic process, abundant during tissue repair. This work demonstrates a critical role of miR-21 in such conversion of macrophages to fibroblast-like cells at the wound site.

K2.03

Viable Cryopreserved Umbilical Tissue (vcut) Barrier Reduces Post Operative Adhesions In A Rabbit Abdominal Adhesion Model

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Background: Postoperative adhesions are common complications in a wide range of surgeries, including abdominal, cardiothoracic and orthopedic procedures. Adhesions, caused by fibrosis, lead to pain and impaired organ function, and often require additional interventions. Fresh placental tissue has been reported to exhibit anti-fibrotic and anti-inflammatory properties, however tissue preservation methods have been shown to alter placental components and negatively impact tissue properties. Recently viable cryopreserved umbilical tissue (vCUT) allograft, with all tissue components preserved in their native state, became available for clinical use. We hypothesized that the application of vCUT would prevent the development of postoperative adhesions. Methods: To test our hypothesis we used a rabbit abdominal adhesion model. Cecum surface adjacent to sidewall defect and facing the rest of bowel was abraded with a sterile gauze for 15minutes and 5minutes respectively until petechial hemorrhage was observed. A 3x3cm^2 of peritoneum and abdominal transverse muscle was removed on the lateral abdominal wall. vCUT was sutured to abdominal wall in test animals, followed by the bowel replaced into the abdomen and closure of cavity in all animals. Days 7, 28 and 74 post-surgery animals were euthanized and visually inspected for the presence of abdominal adhesions. Results: There were no detectable adhesions in animals treated with vCUT at all study time-points. Furthermore, histological analysis of peritoneum, cecum, and/or vCUT tissues was performed. Adhesion, inflammation, and fibrosis were scored on a scale of 0-4. Scores were lower at days 28 and 74 in the vCUT group versus the control group (1.79 ± 0.89, $p<0.0001$, $n=8$). Although degraded over time, vCUT on the peritoneal wall persisted up to 74days providing adequate time for surgical wounds to heal. Conclusions: vCUT decreased formation of postoperative adhesions *in vivo* in a rabbit abdominal adhesion model. This data supports the use of vCUT as an adhesion barrier in surgical procedures.

K2.04

The Role Of The Microsurgical Tissue Transfer In Diabetic Foot Ulcer: Completing The Most Functional Healing

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Background: The majority of diabetic foot ulcer (DFU) patients have occlusive peripheral arterial disease, many microsurgeons tend to hesitate performing free flap for DFU reconstruction, especially, when the occlusive peripheral arterial disease (PAD) is complicated. Nowadays, the application of free flap for DFU is increased and high success rate is reported, however, it has not been universal. We would like to notice our results and discuss the role and worth of the free flap in the DFU reconstruction. Methods: From September 2010 to August 2017, 171 free flap operations were performed for 168 patients. All ulcers were invaded into bony level through fascia and tendon with infection. The preoperative PTA was done if indicated and every patient was fully evaluated for medical and anesthesiological problem. After completion of wound bed preparation, the free tissue transfer was performed. Results: Preoperative PTA were done in 116 patients(68%) and 17 patients (10%) had end-stage renal disease (ESRD). 141 flaps of 171 flaps showed complete flap survival (82%) and 11 flaps were partially necrotized. Overall flap survival rate was 89%. Among 19 total flap necrosis, 10 flaps were replaced with skin graft, six were healed with dressing, and three were treated with below knee amputation (BKA). With exception of patients treated with BKA, the other patients are able to walk with own their feet and satisfied very highly in both terms of function and aesthetics. Conclusions: Our results showed overall success rate of 89 % that is not too low to look away free flap in DFU. The preoperative PAD and ESRD seemed to have negligible value on free flap survival in DFU. Because most of DFU occur on the plantar where has a role for weight bearing, free flap is more superior to other reconstruction method like skin graft or secondary healing. According to our results and experiences, I would like to highly recommend free flap for DFU for the best functional and aesthetic outcomes.

K2.05

Substance P Activates The Epidermal Dendritic T Cells To Promote Wound Healing By Producing Ngf

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BACKGROUND: Accumulating evidences have show that neuro-immuno-endocrine modulation plays an essential role in the wound healing. However, the underlying mechanisms behind this axis still remain unknown. Here, the aim of this study is to clarify the role of substance P on the dendritic epidermal γδ T cells (DETC) in the wound healing. METHODS: The epidermal dendritic epidermal T cells (γδT cells), which were isolated and then further sorted by FACS, 7-17 T cells and PAM212 cell line were used here. After treatment by substance P, a series of methods (ELISA, CCK8 assay, RT-PCR, western blotting, scratch wound healing) were applied to analyze. RESULTS: Neurokinin receptors (NKRs) were not only expressed in skin γδT cells, but also located with differently subtypes in epidermal and dermal skin γδT cells. Following substance P treatment, it rapidly activates γδT cells and leads to an increase CD69 expression, and NGF production, and to changes in DETC morphology (p<0.05). Furthermore, we find that TCR activation is necessary for substance P-induced NGF production in γδT cells. In addition, substance P-activated γδT cells not only enhance keratinocytes migration, but also increase enhance keratinocytes proliferation (p<0.05), which inhibited by NK1R inhibitor and NGF inhibitory antibody. Finally, we demonstrated that substance P-induced NGF production by γδT cells does require ERK1/2 activation (p<0.05). CONCLUSIONS: Here, we uncover a novel mechanism of neuro-immuno-endocrine modulation based on the fact that substance P can activate γδT cells induces NGF production. These findings suggest that it could be used in the therapeutically in the clinical wound treatment.

K2.06

Human Macrophage Response To Bacterial And Fungal Isolates From Diabetic Foot Ulcers

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BACKGROUND - In diabetic foot ulcers (DFUs) the wound environment is in continuous contact with colonizing microbes, which have potential to modify healing responses and/or cause clinical infection. Macrophages, the primary innate immune cells, kill infiltrating pathogens and modulate the local micro-environment. As modulators of their environment, macrophages are multifunctional, interacting with their environment and eliciting changes their behavior to regulate the diverse processes of wound healing. METHODS - To better understand the response of macrophages to the colonizing microbiota, conditioned media from bacterial and fungal isolates from DFUs were added to primary human macrophages for 24 hours, *in vitro*. Analysis of macrophage gene expression and protein production showed that both markers of pro-inflammatory M1 and wound-resolving M2 phenotypes were upregulated to different extents by five bacterial and one fungal species. RESULTS - Specifically, *Corynebacterium amycolatum*, *Corynebacterium striatum*, and *Pseudomonas aeruginosa* conditioned media promoted macrophages to increase secretion of PDGF-BB, a cytokine involved in ECM deposition along with pro-inflammatory cytokines VEGF, IL1β, and TNFα, relative to unactivated M0 and M1 macrophage controls, collectively suggesting a fibrotic response. CONCLUSION - Therefore, these data indicate irregularities in macrophage behavior to different colonizing microbes, suggesting that the microbiome of patients could be used as an indicator for tailored chronic wound treatments. Investigation of the unique responses of macrophage to colonizing microbiota could be used to engineer chronic wound solutions that address the macrophage deficits.

WHS SESSION K: Concurrent Session: ECM, Fibrosis and Scarring
Friday, April 27, 2018 2:15 P.M. - 3:15 P.M.

K3.01

Role Of Mrtf-a And Mrtf-b In Post-operative Intra-abdominal Adhesion Formation

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Background: Post-operative intra-abdominal adhesions occur in over 90% of patients who undergo abdominal surgery. Intra-abdominal adhesion scar tissue can cause significant morbidity and mortality. Myocardin-related transcription factors-A and -B (MRTFs) regulate myofibroblast function in cutaneous wound healing. Similar to cutaneous wounds, intra-abdominal adhesions are enriched in myofibroblasts and collagens. We hypothesize that myofibroblast-mediated formation of intra-abdominal adhesions require MRTFs. Methods: Intra-abdominal adhesion tissue was collected from patients undergoing a secondary surgery. Half the tissue was fixed and embedded in paraffin. Sections were immunostained for MRTF-A, MRTF-B, smooth muscle α-actin (SMαA), and collagen type III. Cells were isolated and cultured from the other half of tissue using Liberase TM. Cytoplasmic and nuclear extracts from patients' cultured cells were subjected to Western-immunoblotting for MRTF-A, MRTF-B, Tubulin, and Lamin A/C. Patients' cultured cells were infected with lentiviral vectors expressing either shRNA targeting MRTFs or non-targeting shRNA. Lysates from infected cells were collected and subjected to Western-immunoblotting for MRTF-A, MRTF-B, SMαA, SM22α, collagen type III, and GAPDH. Contractile properties of infected cells were analyzed in a stressed-relaxed collagen lattice contraction assay. Results: MRTFs are highly expressed in adhesion tissue and correlated with expression of SMαA and collagen type III. MRTFs were found localized in the nucleus in all cultured patient adhesion cells and correlated with the expression of the pro-contractile genes (SMαA, SM22α) and pro-fibrotic collagen type III. shRNA mediated depletion of MRTFs reduced expression of these myofibroblast genes. Lastly, depletion of MRTFs reduced stressed-relaxed collagen lattice contraction. Conclusion: Presence of MRTFs correlates with the presence of myofibroblasts in adhesion tissue, while depletion of MRTFs in cultured patient adhesion fibroblasts decreases the functional characteristics of myofibroblasts. Animal studies are currently underway to directly test whether inhibition of MRTFs prevents the formation of myofibroblasts and of intra-abdominal adhesions. Therapeutic approaches to inhibit MRTFs may be useful in preventing post-operative adhesion formation.

K3.02

Regulation Of Hyaluronan Metabolism Attenuates Organ Fibrosis

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Background: Organ fibrosis is a common disease endpoint that confers high morbidity. We and others have reported that skin injury alters hyaluronan (HA) metabolism, and that HA regulation by IL-10 or HA-synthase(HAS1) encoding for anti-inflammatory HA subtypes promotes regenerative wound healing. Here, we postulate that HA dysregulation is central to fibrosis and further hypothesis that the regulation of altered HA metabolism attenuates renal fibrosis. Methods: Dermal and renal fibroblasts (FB) were isolated from C57BL/6J mice to determine the effect of IL-10(100 ng/ml) with/without TGFb (to induce a fibrotic phenotype), and/or the HA inhibitor, 4-methylumbelliferone(4MU), on expression of HAS1-3 and hyaluronidases (HYAL1-2). We performed unilateral ureteral obstruction(UUO) as a renal fibrosis model with/without IL-10 or HAS1($1x10^{10}$IU) overexpression through the injection under the kidney capsule. UUO/sham kidneys were collected at d3, d7, d14 for RNA, ELISA, and immunohistochemistry. HA metabolism levels were assessed by qPCR, Western blot, and ELISA. HA molecular weight was assessed by gel electrophoresis. Data mean+/-SD; p-values by ANOVA. Results: *In vitro:* TGFb significantly upregulated gene expression of collagen-1($p<0.05$) in both FB cell lines, as expected; expression of HAS1-3 in skin FB (3.23±0.37, 1.73±0.23, 5.25±1.10), and HAS1&3 and HYAL1 in renal FB (6.62±0.89, 1.83±0.54, 1.84±0.92) was also significantly dysregulated ($p<0.05$). 4MU treatment abrogated TGFb-induced fibrotic phenotypes in both FBs. IL-10 restored HAS gene levels in both FBs (1±0.42 fold). *In vivo:* UUO significantly altered HA synthase and hyaluronidases, resulting in dysregulated HA metabolism. HAS gene expression levels spiked at d7 in UUO and HYAL1 increased 6-fold at d3 ($p<0.05$), while HA expression peaked at d14 (4.72x103 ng/ml). IL-10, but not HAS1, regulated post-translational levels of HA, attenuated scarring, and reduced α-SMA relative to controls. Conclusions: Our findings demonstrate that regulation of HA metabolism can attenuate post-injury fibrosis in multi-organ fibrosis models, identifying a potential common biology. Moreover, our discovery of mechanisms behind the HA-attenuated fibrosis could inspire novel therapeutics.

K3.03

Comparative Rna-seq Transcriptomic Analysis Using Ingenuity Pathway Of Unscarred Human Skin, Versus Normal Scarring And Abnormal Keloid Scars

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BACKGROUND Successful treatment of pathological keloid scarring is an unmet clinical challenge due to their elusive pathobiology. Keloids are fibroproliferative dermal lesions resulting from deep dermal injury and occur exclusively in humans. They exhibit quasi-neoplastic traits amongst a myriad of other characteristics although it is not clear how they are related. Based on the central dogma of biology, we hypothesize that a comprehensive exploration of the keloid scar transcriptome can reveal novel biological patterns to explain its pathobiology in relation to normal scarring and skin. Current studies on the keloid transcriptome are based on PCR and microarray studies, which are limited in their dynamic range. METHODS To approach our hypothesis, we performed comparative transcriptomic analysis of the human skin, normal scar and keloid tissues using RNA-seq. This was conducted using Illumina HiSeq 4000 for 8 skin (C), 4 scar (S) and 11 keloid (K) samples obtained from 20 patients, resulting in pairwise comparison across 3 groups: keloid versus skin (KC), scar versus skin (SC) and keloid versus scar (KS). RESULTS We identified 7120, 2748 and 150 differentially expressed genes (p-adj<0.05) in KC, SC and KS respectively using DESeq2. Further exploration of these genes using Gene Ontology and Ingenuity Pathway Analysis revealed unprecedented gene signatures alongside biological processes and pathways, which may contribute to the unique phenotype of the disease. We confirmed the presence of dysregulated extracellular matrix metabolism, which is underlined by processes relating to increased cell survivability. In particular, we highlighted the putative role of a dysregulated mechanism in keloids encompassing transcription, translation and post-translational modifications of proteins. CONCLUSIONS This is the first comprehensive study of the keloid transcriptome using RNA-seq. It confirms our hypothesis and sheds new insights into keloid pathobiology, supporting the identification of candidate biomarkers and pathways for further study geared towards better therapeutic development.

K3.04

WT1Transcripts Are Alternatively Spliced And M1 Cytokine Inducible In Palmar Fascia Fibroblasts

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Background: Excessive fibroproliferation and matrix deposition into palmar fascia, known as Dupuytren's disease (DD), can induce debilitating palmar-digital contractures. We have previously reported upregulated expression of *WT1*, an oncogene with roles in cancer progression and epithelial-mesenchymal transition, in DD. In tumors, alternative splicing of *WT1* gene transcripts results in WT1 isoforms that act as transcription factors or RNA splicing factors. The roles of WT1 proteins in palmar fascia repair are currently unclear. Methods: Total *WT1* transcript levels in primary fibroblasts derived from fibrotic palmar fascia (DD fibroblasts), syngeneic "pre-fibrotic" (PF) fibroblasts and allogeneic normal control (CT) palmar fascia fibroblasts were assessed by qPCR, whereas alternatively spliced *WT1* transcripts were identified by Reverse Transcription-PCR. "Cytomix" treatments with pro-inflammatory (M1) cytokines (TNF, IL1ß and IFN gamma) were used to mimic the early (M1) phase of tissue repair. Alamar Blue assays were used to assess the proliferation of CT fibroblasts transduced with adenoviral constructs expressing *WT1* splice variants or GFP (vector control). RNA sequencing analyses of adenoviral gain-of-function and CRISPR-mediated loss-of-function constructs are underway. Results: Unlike PF and CT fibroblasts, DD fibroblasts constitutively express alternatively spliced *WT1* gene transcripts. Although *WT1* RNA expression is normally low in PF and CT fibroblasts, pro-inflammatory (M1) cytokines induce the expression of (at least) 4 different alternatively spliced *WT1* gene transcripts. Adenoviral expression of WT1 isoform B, predicted to be a transcription factor, promotes the proliferation of CT fibroblasts relative to vector-transduced controls. Conclusions: These findings reveal abnormally sustained expression of alternatively spliced *WT1* transcripts in DD, and implicate roles for transient expression of *WT1* isoforms during the early inflammation phase of normal palmar fascia repair. We anticipate that our ongoing gain-of-function and CRISPR-mediated loss-of-function analyses will identify novel WT1 targets with roles in the normal and abnormal repair of palmar fascia, and potentially, in other WT1-positive tissues or organs fibroses.

K3.05

Potential Role Of Neuropeptide Receptors In Scleroderma

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PURPOSE:

Scleroderma(SSc) is collagen-vascular-disease that manifests initially with dermal-fibrosis, then progresses to multiple-organ-fibrosis. No treatment to arrest SSc. Recently, mouse-model of SSc was reported, in which Bleo is injected intradermally. Reactive-oxygen-species (ROS) is associated with dermal-fibrosis. We previously demonstrated that ROS trigger GRP-mediated pulmonary-fibrosis to hyperoxia or radiation. Now we test the hypothesis that gastrin-releasing-peptide(GRP) from cutaneous-nerves has role by activating myofibroblasts[alpha-smooth muscle actin, SMA+] and pericytes[SMA+ and neural/glial antigen 2, NG2+] utilizing drugs and blocking-antibodies. We tested expression of both GRP-receptors, GRPR and neuromedin-B receptor (NMBR) by immunohistochemistry.

METHODS:

Flanks of 10-wk-old C3H/HeJ mice were injected intradermally with Bleo(5d/wk for 3-wks). Mice also received antioxidant N-acetylcysteine(NAC) IP, and GRP-blocking mAb-2A11. After 21d, lesions were immunostained for SMA,NG2,GRPR,NMBR. Immunostaining in dermis and epidermis was scored on scale from(0-3), comparing prevalence of(+)cells.

RESULTS:

Bleo induced>10-fold-increase in pericytes & myofibroblasts, in dermis($P<0.001$), NG2 and SMA staining scores were linearly correlated($R^2=0.87$,$P<0.05$). SMA & NG2 were reduced by NAC(~80% decrease,$P<0.001$) or mAb2A11(~50% decrease,$P<0.01$), similar to prior studies of dermal-thickness. Epidermal-scores for GRPR were significantly decreased in Bleo+2A11 mice compared to Bleo alone(0.5 ± 0.3, 1.9 ± 0.3, $P<0.005$), like prior studies of GRPR up-regulation by GRP. However, there were no other differences in GRPR between groups. NMBR scores were unchanged.

CONCLUSION:

Increased pericytes and myofibroblasts occur in regions of dermal-fibrosis. Although GRPR &/or NMBR could contribute to dermal-fibrosis their expression is unchanged. GRP induces GRPR gene-expression. Sustained-epidermal-expression of both receptors would be consistent with potential GRP-signaling in epidermis as mechanism for epidermal-hyperplasia and dermal-fibrosis, such as through epithelial-mesenchymal-transformation.

K3.06

Myofibroblast Differentiation Of Fetal Fibroblasts Is Inhibited In Response To Ecm Rigidity And Tgf-b1

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During dermal wound healing, fibroblasts differentiate into myofibroblasts and excessively contract and remodel newly deposited extracellular matrix (ECM) leading to scarring. Myofibroblast differentiation is driven by biomechanical factors in the wound environment including ECM rigidity and transforming growth factor-b1 (TGF-b1). These environmental factors promote the formation of mature focal adhesions and stress fibers rich in a-smooth muscle actin (a-SMA) that generate the large contractile forces necessary for wound closure. In contrast, fibroblasts orchestrate scarless healing of fetal dermal wounds with minimal myofibroblast involvement or contraction suggesting that smaller cellular forces contribute to this process. However, it remains unclear whether this regenerative response is a result of unique biomechanical characteristics of fetal fibroblasts or their wound environment which lacks TGF-b1 and is composed of a more compliant ECM than found in adult wounds. Therefore, we tested whether physiologic rigidities and TGF-b1 promote actomyosin contractility and myofibroblast differentiation in fetal fibroblasts using a fibronectin-polyacrylamide gel (PAA) system that spanned the mechanical properties reported for different wound healing stages. We found that focal adhesion formation and/or maturation was impaired in fetal fibroblasts at early and late time points on rigid PAAs that mimicked late-stage granulation tissue when compared to adult fibroblasts. These differences coincided with less traction force generation by fetal versus adult fibroblasts on these PAAs. Furthermore, TGF-b1 did not induce myofibroblast differentiation of fetal fibroblasts on rigid PAAs in comparison to adult fibroblasts which exhibited increased focal adhesion formation and maturation, a-SMA levels, and traction forces. Overall, our data suggest that fetal fibroblasts have inherently different biomechanical responses to environmental factors resulting in a unique contractile phenotype that may prevent myofibroblast differentiation.

WHS SESSION K: Concurrent Session: Angiogenesis
Friday, April 27, 2018 2:15 P.M. - 3:15 P.M.

K4.01

Angiogenesis Through Stimulation With External Volume Expansion Improves Adipose Tissue Graft Retention In A Radiation Fibrosis Model

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Introduction: Post-mastectomy radiation therapy (RT) has improved breast cancer survival but has complicated the breast reconstruction process. Autologous fat grafting (FG) is a novel reconstruction tool used by plastic surgeons. However the hypovascular environment after radiation exposure hampers the retention of adipose grafts. Pre-treatment with External Volume Expansion (EVE) is known to induce angiogenesis through mechanical stimulation and hypoxia. We hypothesized that could improve vasculature in irradiated tissue and improve fat graft retention.
Methods: Forty mice were divided into 4 groups of 10 mice each. 50gy of topical radiation was applied to mice on their flanks and were monitored for 8 weeks until development of chronic fibrosis. Group 1 received unilateral RT. Group 2 received bilateral RT and EVE application for 5 days unilaterally with 25mmHg of negative pressure. Group 3 (n=10) was not irradiated and received human fat graft as control. Group 4 received bilateral RT application, then EVE as group 2, followed by bilateral fat grafting. Skin perfusion was measured using Hyperspectral Imaging. Fat graft volumes were quantified 8 weeks post-grafting using CT scans. Histology of tissues was analyzed for vascularity (CD31) and cell proliferation (ki67).
Results: Group 1. Irradiated skin was less perfused compared to control side.
Group 2. EVE application induced a 37% increase in vascularity in the overlying skin and a 45% increase in proliferating cells in skin in the RT treated areas compared to RT without EVE.
Group 3 and 4. Fat graft retention after 8 weeks was 74% in the control group, 66% on irradiated and 79% on irradiated and EVE preconditioned group.
Conclusions: Radiation injures microvasculature and reduces skin perfusion affecting fat graft survival. EVE increased volume retention of fat grafts likely via angiogenesis and mechanotransductive preconditioning phenomena.

K4.02

Interleukin-10 Improves Diabetic Wound Neovascularization Via Endothelial Progenitor Cell (epc) Recruitment

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Introduction: Deficiencies in EPC recruitment and function are well-documented in chronic diabetic wounds. The anti-inflammatory cytokine IL-10 was shown to contribute to neovascularization in a murine myocardial infarction model. We report a novel role for IL-10 in promoting neo-angiogenesis in normal and diabetic wound healing by testing the hypothesis that IL-10 promotes EPC recruitment from bone marrow to dermal wounds by regulating stromal-cell derived factor 1(SDF1-alpha) and vascular endothelial growth factor(VEGF) via a STAT3-dependent mechanism. Methods: We evaluated the impact of IL-10 overexpression(lentiviral IL-10 $1x10^6$ TU) in WT and db/db-diabetic wounds(n=4/group): 4mm stented wounds were analyzed at day7 for EPCs(CD133+/Flk1+cells/HPF; confocal microscopy), neovascularization(Meca32+vessels/HPF), and wound healing/inflammation (H&E;F4/80+cells/HPF). Skin-specific, tamoxifen-inducible STAT3 knockout mice modelled the mechanism of EPC mobilization and angiogenesis: circulating EPCs(CD34+/CD133+/Flk1+ cells;FLOW-cytometry) and VEGF and SDF1-alpha in bone marrow, serum and wounds(ELISA;qPCR;IHC) were quantified at day3. In vitro, WT dermal fibroblasts(FB) treated with IL-10(200 ng/ml) were analyzed for VEGF and SDF1-alpha. The effect of IL-10 and IL-10-treated FB-conditioned media on angiogenesis were tested in aortic ring assay. Data presented as mean±SD;p-values by ANOVA. Results: At day7, lenti-IL-10-overexpression significantly improved wound healing, EPC expression($p<0.01$), and neovascularization($p<0.001$) while significantly reducing macrophage abundance($p<0.001$) in normal and diabetic wounds. In WT-mice, lenti-IL-10-overexpression significantly increased circulating EPC levels at day3 post-wounding(2.7±0.5fold;$p<0.001$), which was associated with increased SDF-1alpha expression in serum/wounds and decreased in marrow($p<0.01$). Similarly, lenti-IL-10-overexpression at day3 demonstrated increased serum, wound, and marrow VEGF expression($p<0.05$). STAT3-knockdown abrogated the aforementioned day3 and day7 effects of IL-10($p<0.01$). In vitro, IL-10 significantly increased FB production of VEGF($p<0.05$) and SDF1-a($p<0.05$). Relative sprouting area on aortic ring assay increased in IL-10-treated FB-conditioned media as compared to FB-conditioned media alone or media+IL-10 ($p<0.01$), suggesting FB as a potential target cell for IL-10-induced angiogenic effects. Conclusion: IL-10-driven EPC recruitment via STAT3, VEGF, and SDF-1alpha may be an innovative strategy to induce therapeutic angiogenesis and tissue repair in normal and diabetic wounds.

K4.03

Decreased Lymphangiogenesis In The Skin Of Patients With Keloid

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BACKGROUND: Vascular abnormalities are one of the primary pathological components of keloid. However, it has not been determined if there are also abnormalities in the formation of lymphatic vessels in keloid. The aim of this study is to evaluate lymphangiogenic activity in keloid skin. METHODS: Skin biopsies were collected from the involved skin of 11 patients with 11 and from the skin of 6 healthy volunteers. The numbers of D2-40, LYVE-1 and podoplanin -positive lymphatic vessels in skin specimens from healthy control subjects and patients with keloid were counted and compared. Quantitative real-time polymerase chain reaction (PCR) was performed to determine mRNA levels of the various splice variants of vascular endothelial growth factor, VEGF-A, VEGF-C and VEGF-D, their receptors VEGFR1, VEGFR2 and VEGFR3, TGF-β1, PROX1, LYVE1, podoplanin, bFGF. RESULTS: The number of lymphatic vessels in patients with keloid was significantly decreased compared with healthy control subjects ($p<0.05$). Mean relative transcript levels of FIGF (VEGF-D) and VEGFR3 in skin tissue from patients with keloid were significantly increased compared with healthy control subjects ($p<0.05$). Conclusions: A systemic increase of VEGF-D, as well as local overexpression of VEGFR3, may be the cause of disturbed lymphangiogenesis in keloid skin and play a role in the pathogenesis of keloid. We showed the possibility that regulation of VEGF-D／VEGFR3 signalling could lead to new treatment of skin ulcers in the keloid by controlling the formation of lymphatic vessels.

K4.04

Improved Perfusion and Wound Healing in Healthy Pigs With MRG-110, an Inhibitor of MicroRNA-92a

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miR-92a has previously been shown to inhibit angiogenesis. Conversely, inhibitors of miR-92a accelerate angiogenesis, improving function following myocardial infarction and vascular injury. The current study suggests miR-92a inhibitors can accelerate wound healing in normal healthy skin as well. The effect of a miR-92a inhibitor on wound healing was investigated in a GLP pig wound healing study. Vehicle or 3 dose levels of a locked nucleic acid (LNA)-modified inhibitor of miR-92a, MRG-110, were administered three times a week for two weeks by intradermal injection around the periphery of 2.5 x 2.5 cm full thickness excisional wounds. Treatments were randomized across eight wound sites on each of six pigs (12 wounds/MRG-110 dose level). Two control pigs with 8 wounds each were treated with either standard of care (SOC; wound dressing changes only) or phosphate buffer vehicle control. Wound healing and perfusion were assessed by photography, wound area measurements, laser Doppler imaging, and histology over a 7-week period until complete wound closure was achieved. MRG-110 significantly ($p<0.05$) increased vascularization within the dermal portion of the wound bed which was associated with significant ($p<0.05$) increases in perfusion in drug treated wounds compared to controls on Day 14. Additionally, MRG-110 significantly ($p<0.01$) increased granulation tissue formation. These effects led to a ~5-day improvement in achieving 50% wound closure in healthy pigs compared to SOC or vehicle. The lowest dose tested had maximal benefit indicating even lower doses may be effective. These data suggest that MRG-110 has the potential to accelerate wound healing in healthy skin and provide support for future clinical trials of antimiR-92a therapeutics in acute wound healing indications.

K4.05

Epithelial Hypoxamir Mir-210 Directly Contributes To Ischemic Skin Injury

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BACKGROUND - Chronic wounds are commonly associated with peripheral vasculopathies. Limitations in the ability of the vasculature to deliver O_2-rich blood to the wound tissue leads to, among other consequences, hypoxia. Thus, hypoxia is a subset of ischemia. Hypoxia inducible microRNAs, or hypoxymiRs play a significant role in determining outcomes following ischemic insult. miR-210 is widely regarded as a master hypoxymiR. METHODS - To determine the significance of keratinocyte specific miR-210 during ischemic injury, an animal model with keratinocyte specific knockout of miR-210 ($K14^{cre}miR\text{-}210^{\Delta/\Delta}$) was developed by crossbreeding mice carrying floxed miR-210 allele ($miR\text{-}210^{fl/fl}$) with tamoxifen inducible K14-Cre mice. A mono-pedicle flap was developed on the back of the mice by making 30-mm-long full-thickness parallel incisions 10 mm apart. Flap edges were cauterized and then sutured to the adjacent skin. Epithelial keratinocytes were collected using Laser Capture Microdissection (LCM) from the skin flap. RESULTS - Significant knockdown (~50%) of miR-210 was noted in the skin epithelium of the $K14^{cre}miR\text{-}210^{\Delta/\Delta}$ mice compared to their wildtype littermates. Using mono-pedicle model of graded ischemia, induction of miR-210 was dependent on the extent of lack of blood flow at d3 post-surgery. The extent of ischemia was categorically characterized by dividing the flap into three parts (proximal, intermediate and distal). The level of ischemia gradually increased from the proximal to the distal part. Similar finding was observed for miR-210 abundance in LCM-captured epithelium pointing towards the involvement of keratinocytes. Interestingly, miR-210 was elevated, potentially contributed by inflammatory cells, in the distal region of the flap in the K14cremiR-210Δ/Δ. Furthermore, K14cremiR-210Δ/Δ mice showed increased perfusion 3 days after mono-pedicle ischemic flap surgery compared to that of the wildtype. Such advantage in blood flow caused flap survival in K14cremiR-210Δ/Δ mice. CONCLUSIONS - Inhibition of keratinocyte specific miR-210 in the ischemia-affected limb is an effective therapy strategy to improve ischemic chronic wound outcomes.

K4.06

Detection Of Acute Vascular Occlusion Using Oxygen Monitoring In Myocutaneous Flaps

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PURPOSE:

Vascular-compromise occurs in immediate-postoperative period due to failure of micro-vascular-anastomosis. It is estimated that 6-25% of skin flaps require secondary-surgical re-exploration and ~10% of flaps fail. Currently, all monitoring methods have limitations because they require an experience, calibration-difficulties and expensive. Furthermore, these methods impose significant delay between time of vessel-occlusion and detection. We introduce implantable-oxygen-sensors as new method to detect vascular-occlusion.

METHODS:

Experimental-sensors were made by incorporating benzo-porphyrin into matrix of biocompatible-hydrogel. Sensors were approximately 3mm-long1.5mm-wide,0.5mm-thick. Sprague-Dawley rats were used throughout study. Sensors were implanted intradermally in impending flap site. Inspired oxygen was modulated between 100%-12% to qualitatively confirm sensor sensitivity. Superficial inferior epigastric artery(SIEA) myocutaneous-flaps were surgically elevated. Vessels were carefully dissected to create 3×5cm-island-flap containing skin, subcutaneous-fat. Tissue-oxygen tension(TOT) readings obtained from implanted-sensors both at baseline and during vascular-clamping of feeding-blood-vessels.

RESULTS:

TOT-measurements from sensors were observed to modulate correlating with changes in inspired-oxygen levels. Clinical-observation of flaps did not show any significant change in color and temperature of flaps during or immediately after clamping of feeding-blood-vessels. Real-time-analysis of sensors implanted in myocutaneous-flaps has demonstrated that acute-vascular-clamping of feeding-blood-vessels in pedicle were immediately detected within 70sec ($*p<0.05$).

CONCLUSION:

Oxygen-monitoring in tissues is highly-sensitive and can be specific for detection of acute-vascular-occlusion. This is superior to clinical observation, faster than current standard-of-care methods and offers cost-effective, and accurate-means of monitoring free-tissue-transfers.

WHS Session M: Rapid Fire Poster Talks
Friday, April 27, 2018 6:45 P.M. - 7:15 P.M.

M1.01

Protease-activated Receptor-2 Knockdown Attenuates The Fibrotic Phenotype In Post-burn Hypertrophic Scar Fibroblasts

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BACKGROUND: Hypertrophic scarring (HTS) following major burn injury remains a critical morbidity with limited treatment options that ultimately diminishes quality of life for burn victims. We and others have found that cutaneous mast cells (MC) are increased significantly in burn wounds and HTS. Tryptase, released by the stimulated MC, activates the protease-activated receptor-2 (PAR2) and is thought to play an important role in post-burn HTS pathophysiology. Here, we examined serum tryptase concentrations in a pediatric burn population. Additionally, in an *in vitro* model, we investigated the anti-fibrotic effects of PAR2 knockdown in primary post-burn HTS fibroblast cultures. METHODS: Serum was collected from age-matched non-burned and burned pediatric patients with greater than 20% of total body surface area burned in this IRB-approved study. Tryptase-β2 was measured by ELISA. *In vitro*, primary HTS fibroblasts were treated with siRNA to PAR2 or scrambled siRNA for 72 hours prior to 1-hour treatment with PAR2 activating peptide SLIGKV. After treatment, mRNA targets of PAR2 activation and subsequent fibrotic signaling were quantified by RT-qPCR in three independent experiments. RESULTS: Following severe burn injury, serum tryptase increased significantly during acute ICU admission (n=5, 6.104 ng/mL ± 0.93, p=0.0002) and remained elevated for at least 6 months post-burn (n=5, 5.105 ng/mL ± 1.793, p=0.0009) compared to non-burned controls (n=6, 0.7083 ng/mL). Furthermore, PAR2 siRNA knockdown significantly reduces mRNA transcript expression of fibrotic genes, including Collagens-1, and -3, MMPs-2, and -9, and α-SMA ($p<0.05$) compared to control-treated cells. Moreover, stimulation with SLIGKV confirmed complete PAR2 knockdown as there was no increase in the expression of the fibrotic genes. CONCLUSION: Together, these data show PAR2 activation intensifies the HTS fibrotic phenotype in burn HTS fibroblasts and further suggests that targeted PAR2 antagonism at wound sites may decrease MC tryptase's proliferative effect and potentially limit detrimental fibrosis in post-burn HTS over time.

M1.02

Mechanical Education In Vitro Enhances Regenerative Capacities Of Human Mesenchymal Stem Cells In Vivo

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Background: Mesenchymal stem cell (MSC) expansion is crucial to obtain sufficient cell numbers for tissue repair therapies. Traditional culture expansion on stiff surfaces reduces regenerative potential and jeopardizes therapeutic outcomes by inducing scar features in MSCs. In contrast, 'priming' on skin-soft culture surfaces preserves regenerative MSCs that improve the healing of rat wounds (*Li et al. Nature Materials 2017*). We hypothesize that mechanical priming in culture will alter secretory functions and thereby paracrine actions of MSCs in the wound environment. Methods: Human umbilical cord perivascular cells (HUCPVCs) from three donors were soft- or stiff-primed. Mechanical priming was verified using qRT-PCR and Western blotting for markers of stromal cell activation. Conditioned medium from primed HUCPVCs was analyzed using cytokine arrays. Primed HUCPVCs were transplanted to splinted rat full thickness wounds, traced for grafting success, and wound tissue was analyzed for cell and matrix composition. Results: Soft-primed HUCPVCs exhibited faster doubling times (3.0±0.4 vs 4.7±0.4 days) and reduced gene and protein expression of scar markers α-SMA and ED-A fibronectin compared to stiff-primed HUCPVCs. Of 79 cytokines detected in conditioned medium, 48 were down-regulated and 4 were up-regulated in all soft- versus stiff-primed HUCPVCs. Of 10 most differentially expressed cytokines, 7 were related to inflammation and 3 to cell cycle regulation. Soft- and stiff-primed HUCPVCs produced distinct healing outcomes compared to vehicle controls in a rat model of exacerbated wound healing. Conclusions: Soft-priming enhances the regenerative capacity of human MSCs by preserving cell proliferation, suppressing scarring features, and creating distinct paracrine profiles that persist in a wound environment.

M1.03

Fibroblast Mediated Nax Signaling Drives Inflammation In Open Wounds

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BACKGROUND The skin provides a barrier between the outside environment and the internal milieu of the body. Disruption of the stratum corneum which occurs in wounding leads to evaporative water loss and we hypothesize an increase in [Na+]. We have previously shown that when keratinocytes are exposed to a 10% increase in extracellular [Na^+] - which could potentially occur in open wounds, the sodium channel Na_X becomes permeable leading to the influx of Na+. The resultant increase in intracellular [Na^+] in-turn activates signaling pathways leading to inflammation and scar formation. METHODS Fibroblasts are crucial to the wound healing process by stimulating the production of collagen for tissue repair. Fibroblasts may also serve as vital immunoregulatory cells.Me In this study we investigated whether exposure of fibroblasts to increases in extracellular [Na^+] could modulate fibroblast function during wound healing. Here we show that under dehydrating conditions, in non-epithelized wounds where fibroblasts are the predominant cell type, the inflammatory mediators Cox-2 and Il-8 are markedly upregulated. RESULTS *In vitro*, Na_X is highly expressed in fibroblasts and exposure of fibroblasts to a 10% increase in culture media [Na^+] led to a rapid influx of Na^+ and upregulated the expression of COX-2 and IL-8. Knock-down of Na_X expression using shRNA blocked the induction of COX-2 and IL-8 in response to high NaCl. In the presence of increased NaCl collagen contraction and cell migration were not affected suggesting that high Na^+ preferentially modulates the inflammatory signaling pathways within fibroblasts. CONCLUSIONS These data suggest that increases in local [Na^+] such as those that would occur in an open wound stimulate inflammation in fibroblasts via Na_X activation and signaling.

M1.04

Biofilm Infection Poses Risk Of Oxidative Stress Via Redox Cycling Of Secretory Pyocyanin

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Background- While at low concentrations reactive oxygen species (ROS) are known to drive cell signaling towards wound healing, excessive ROS such as during chronic inflammation and diabetes stall wound repair. This work shows that biofilm infection may add to the burden of oxidative stress at the wound site by redox cycling of pyocyanin. Pyocyanin (5-methyl-1-hydroxyphenazine) is a secretory product of *Pseudomonas aeruginosa*. Methods- Engineered human skin was treated with 10μM pyocyanin for 3d. Electron Paramagnetic Resonance (EPR) studies of the treated skin was conducted. The change in reducing equivalents (NADH, NADPH and GSH) pool was also determined in 10μM pyocyanin treated human HaCaT keratinocytes. Results- EPR spectrum of 10μM pyocyanin treated engineered human skin showed the presence of reduced pyocyanin radical which following UV exposure for 30 minutes generated superoxide and hydroxyl radicals. Thus, exposure to direct sunlight can worsen the already damaged biofilm infected wound site. A decline in NADH, NADPH and GSH levels was observed in 10μM pyocyanin treated keratinocytes as compared to untreated cells. Depletion of reducing equivalents was caused by the transfer of electrons from reducing equivalents to pyocyanin which helps bacteria to modulate their intracellular redox state. Modulation of intracellular redox state is crucial for survival of bacteria at high cell densities when there is electron-acceptor limitation, so that they can form thick biofilm. As this is achieved, there is more pyocyanin production, more ROS generation, continuous depletion of reducing equivalents pool and increased oxidative stress which does not allow the biofilm infected wound site to recover. Conclusion- Pyocyanin is cytotoxic to human cells due to its redox-active nature. Such toxicity markedly magnifies upon UV exposure. Pyocyanin oxidizes cellular reducing equivalents, induces oxidative stress and is therefore likely to complicate wound healing.

M1.05

Differential Tight Junction Expression In Skin And Mucosal Wounds

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Background: Oral mucosal wounds heal more rapidly with significantly less inflammation, faster re-epithelialization, more refined angiogenesis, and less scar formation than skin wounds. Tight junctions (TJ) are intercellular junctions between adjacent cells that play pivotal roles in barrier function and cell polarity as well as in innate immunity. Methods: Using microarray, we compared the expression of TJ related genes in mouse skin and tongue wounds at 6, 12, and 24 hours and day 3, 5, 7, and 10 post-wounding. Using qPCR, the expression of occludin, claudins 1 and 4, ZO-1, and JAM-1 was compared between a wounded human skin keratinocyte cell line, HACAT and an immortalized human gingival epithelial cell line, TIGK. Results: The gene expression of multiple TJ molecules including occludin, claudins 1, 3, 4, 7, 10-12, 14, and 23, and ZOs 1-3, and JAMs 1-3 was found to change significantly over the course of healing process in skin wounds. By comparison, tongue wounds showed an overlapping but different pattern of expression that included occludin, claudins 1, 2, 4, 5, 10-13, 15, 18, 19, and 23, and ZO-2 and JAMs 1-3 ($p<0.05$, One-way Anova analysis). Since epithelial cells are one of the primary cell types to express TJ, we next investigated the in vitro gene expression of a subset of the TJ molecules that were differentially expressed in skin and tongue wounds. The expression of occludin and claudin 1 was significantly higher in HACAT than in TIGK after injury; Claudin 4 and JAM-1 were significantly higher in TIGK than in HACAT. No significant difference was observed in ZO-1 expression between these two cell lines after injury. Conclusions: The results suggest that certain TJ molecules are differentially expressed in skin and oral tissues. This differential expression may contribute to the distinct healing phenotypes seen between skin and oral mucosal wounds.

M1.06

Could -79 °C Spray-type Cryotherapy Be An Effective Monotherapy For The Treatment Of Keloid?

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We evaluated the clinical efficacy of our -79 °C spray-type cryotherapy with molecular and pathologic evidence for the treatment of keloids. We evenly split each of ten keloid lesions into a non-treated (C-) and treated (C+) area; the C+ area was subjected to two freeze-thaw cycles of spray-type cryotherapy using -79 °C spray-type Cryotherapy. This treatment was repeated after an interval of two weeks. The proliferation and migration abilities of the fibroblasts isolated from the dermis under the cryotherapy-treated or untreated keloid tissues (at least 5 mm deep) were compared and pathologic findings of the full layer were evaluated. Molecular analysis revealed that the number of dermal fibroblasts was significantly higher in C+ group as compared with C- group. The dermal fibroblasts from C+ group showed more than two-fold increase in the migration ability as compared with the fibroblasts from C- group. The expression of matrix metallopeptidase 9 was increased by more than two-fold and a significant increase in transforming growth factor beta 1 expression and Smad2/3 phosphorylation level was observed in C+ group. C+ group showed more extensive lymphoplasmacytic infiltration with thicker fibrosis and occasional "proliferating core collagen" as compared with C- group. Thus, -79 °C spray-type cryotherapy is ineffective as a monotherapy and should be used in combination with intralesional corticosteroids or botulinum toxin A for favourable outcomes in the treatment of thick keloids.

M1.07

Raman Spectroscopy And Hplc: In Vivo And Ex-vivo Validation Of A Combi-approach For Testing Transdermal Delivery Of Compounds In Wounds And Scars

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BACKGROUND The goal of any topical formulation is efficient transdermal delivery of its actives. However, delivery of compounds can be problematic with penetration through layers of dermal scar tissue. Testing of the presence and depth of penetration of compounds can prove challenging. We propose a new combined approach to assessment of transdermal delivery of topicals; initially using an ex vivo human skin culture with high performance liquid chromatography (HPLC), and subsequently validated by Raman Spectroscopy (RS) of in-vivo human normal and scarred skin. METHODS Topicals were applied to ex-vivo skin organ culture, which were quantified by an optimised HPLC system. Longitudinal sections were analysed to differentiate presence of the topical between skin layers. In-vivo normal and scarred skin from sequential biopsies with application of both topicals were analysed with RS by acquiring static spectral point measurements from cross sections. RESULTS HPLC isolated peaks for 2 actives. One compound was identified in ex vivo 'whole skin' and within the papillary and reticular dermis. The presence of the topical was confirmed by RS in the epidermis and reticular dermis (98% and 99% specificity, 89% and 93% sensitivity, 96%, and 97% accuracy respectively). RS also demonstrated presence of this topical over sequential time points (day 0 to week 8 and 12). Alterations in the secondary structure conformation of protein peaks (α-helix to β-sheet) accounted for changes during remodelling. CONCLUSIONS This unique approach enables successful detection as well as measurement of exact depth of penetration of compounds following application of a topical formulation in cutaneous scar tissue in both ex-vivo and in-vivo models. Where there is uncertainty regarding discrimination of skin layers using longitudinal sections, the cross-sectional approach in RS can validate findings, allowing for the simultaneous evaluation of the effects of the compounds in healing wounds and scar maturation over time.

M1.08

Contribution Of Individual Satellite Cells To Muscle Regeneration Assessed Using A Confetti Mouse Model

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Background: Satellite cells are capable of completely replacing muscle fibers after injury, by producing a large number of myoblasts. However, the contribution of individual satellite cells is poorly understood in vivo.

Methods: To assess the relative contribution of individual satellite cells, we developed a mouse with inducible fluorescence in satellite cells [1]. After tamoxifen induction, the mice express one of four fluorescent colors in Pax7+ cells, while the remaining muscle tissue is non-fluorescent.

Results: Sixteen days after a cardiotoxin injury in healthy 6-month-old mice, up to ninety percent of muscle fibers in the injured area were fluorescent, indicating that fluorescent satellite cells contributed to regeneration of almost all fibers. Most strikingly, the four fluorescent colors of the confetti mouse appeared in regional patches, rather than uniformly distributed across the fibers. Using computational image analysis [2] and stochastic modeling, we analyzed the distribution of observed isochromatic patches, and concluded that the observed color patches were not statistically compatible with satellite cells contributing myonuclei to only a single fiber. Instead we infer that roughly a third of the fluorescent satellite cells must have contributed myonuclei toward multiple fibers.

Conclusions: These results have implications for our understanding of the muscle regeneration process, not only in normal circumstances, but also in pathologies such as muscular dystrophy, sarcopenia, and chronic ulcers. Furthermore, this confetti mouse will be useful for further studies of muscle regeneration, particularly for interrogating the ability of candidate interventions [3-4] to alter the regenerative function of endogenous satellite cells.

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WHS SESSION N: Concurrent Session: Scarring, ECM & Regeneration
Saturday, April 28, 2018 9:15 A.M. - 10:15 A.M.

N1.01

MechanicalTension Regulates Mesenchymal Stem Cell Paracrine Signaling on DermalFibroblasts via microRNA- and lincRNA-enriched Exosomes

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Background: Mesenchymal stem cells(MSCs) have a huge therapeutic potential in wound healing. Cues from the extracellular environment affect MSC secretome, but the role of mechanical tension on the bioactive extracellular vesicles(EVs), namely exosomes released by MSCs is not known. We hypothesized that mechanical tension regulates MSC exosome production and influences wound healing via paracrine effects on dermal fibroblasts. Methods: Human MSCs were cultured on silicone membranes +/-10% static/cyclic strain for 24h and analyzed for phenotypic changes(alpha-SMA, and inflammation PCR-array) and genes encoding exosome synthesis(RAb27a-b;SMPD3). Exosomes were isolated and analyzed for size and quantity(Zetasizer). The exosome protein level was quantified(BCA Assay) and Westernblotting(CD63,HSP70,CD9) and Next-Gen Sequencing(Exo-RNA) were performed. Exosomes were labeled by Exo-Glow before use in a primary human dermal fibroblast(FB) migration assay. p-values by ANOVA;(n=3/group). Results: Tension induced morphologic changes and increased alpha-SMA staining in MSCs. There was a significant change(>5-fold) in ~ 30/84 inflammatory genes with tension. Tension downregulated the expression of RAb27a-b and SMPD3($p<0.01$) in MSCs, but more exosomes with increase in size distribution and protein levels were produced by tension($p<0.05$). The three exosome surface markers were verified by Westernblotting. Tension induced significant changes in abundance(>100) of several lincRNAs and miRNAs in exosomes, which are being evaluated using ingenuity analysis. MSC-derived exosome uptake by FB was tracked using fluorescent imaging. Interestingly, MSC-derived exosomes under static conditions slowed the migration of FB in a scratch wound assay, whereas those derived under tension increased FB migration($p<0.05$), but there was no effect of the complete MSC-conditioned media from either static or tension conditions on FB migration. Conclusions: Tension induces a fibrogenic and inflammatory phenotype in MSCs. Exosomes are a likely target for extracellular communication, as their production/cargo in MSC are regulated by tension and can influence FB behavior. The novel insight of how tension effects MSC paracrine activity will play a pivotal role in clinical MSC therapies.

N1.02

A Complex Mechanism Of Extracellular Matrix Induction By Er Chaperone Calreticulin And Tgf-β For Tissue Regeneration

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BACKGROUND: A lack of extracellular matrix (ECM) synthesis for neodermal formation is a hallmark of impaired wound healing such as in diabetic foot ulcers (DFUs). We have shown that topical application of calreticulin (CRT) markedly enhances the rate and quality of wound healing in porcine and diabetic mouse models by promoting cellular migration and proliferation of keratinocytes for re-epithelialization, recruitment and anti-microbial action of macrophages, and migration, proliferation, and ECM induction by fibroblasts for reconstitution of the defect. Using CRT null mouse embryo fibroblasts (MEFs), it was previously shown that TGF-β-induction of collagen and fibronectin requires intracellular CRT (iCRT) for expression and processing of these ECM proteins. Since exogenous CRT (eCRT) and TGF-β both induce these same ECM proteins, we hypothesized that CRT might signal protein synthesis of ECM proteins through release of TGF-β. RESULTS: We show that CRT treatment of human neonatal fibroblasts induced TGF-β1/TGF-β3 proteins within 3 hours whereas collagen, fibronectin, elastin, smooth muscle cell actin (αSMA), and α5 and β1 integrins expression was not observed until 24 hours. Furthermore, an inhibitor of TGFβ receptor I signaling, SD208, completely blocked eCRT-mediated induction of these ECM proteins and αSMA but not the α5/β1 integrins in both neonatal and adult dermal fibroblasts thus, suggesting that only the ECM proteins and αSMA require TGF-β signaling. Additionally, CRT-mediated TGF-β1 release into media (ELISA) and ECM protein production, but not α5/β1 integrin were blocked by RAP, an inhibitor of LRP1 signaling. Importantly, eCRT did not induce ECM proteins in CRT null MEFs. CONCLUSIONS: The data supports the following schema: eCRT binds LRP1 receptor, LRP1 signaling induces TGFβ1/3, TGF-β1/3 are released from cells and, in an autocrine manner, bind TGF-β receptors to induce iCRT-dependent induction of ECM proteins. Although ECM protein induction by eCRT involves TGF-β, the responses are attenuated and mimic tissue regeneration without scaring implicating eCRTs strong therapeutic potential for DFUs.

N1.03

Micro-architectural Analysis Of Unscarred And Scarred Human Dermis Provides Structural Insight For Future Scaffold Design

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INTRODUCTION The three-dimensional (3-D) spatial arrangement of dermal tissue plays a crucial role in directing cellular behaviour during wound healing. Thus, it is crucial to elucidate a better understanding of the three-dimensional dermal architecture of the human skin. The aim of this project was to understand the configuration in morphological structure of decellularised human dermis between unscarred skin and normal cutaneous scars. METHODS Skin samples were obtained from ethically consented volunteer patients undergoing Abdominoplasty surgery. All skin samples underwent decellularisation as previously described (DNA removal = 88%.). Histological sections of cellular and decellularised dermis were subsequently analysed using standard haematoxylin and eosin (H&E), and 4',6-diamidino-2-phenylindole (DAPI) stains. In addition, extent of decellularisation was quantified using an Easy-DNA^{TM} isolation kit. Nanomechanical and structural evaluations were performed using Atomic Force Microscopy (AFM) and Multiphoton Microscopy (MPM). RESULTS Interestingly, there was no change in the gross morphology of decellularised unscarred and scarred dermis, under light microscopy. However, MPM and AFM showed that collagen fibers in unscarred decellularised dermis were arranged randomly. Collagen fibers of decellularised unscarred dermis appeared to have a significantly rougher (R_q-16.5, R_a-12.5, R_{max}-198; $p<0.0001$) surface topography. Based on AFM reduced modulus values, collagen fibers of unscarred decellularised dermis were less stiff (mean 2.155 MPa ± 0.9595; $p<0.0001$) compared to decellularised scarred dermis. MPM demonstrated that collagen fibers in unscarred dermis are interwoven, akin to a mesh-like structure. Further, scarred dermis has a higher collagen volume density. CONCLUSIONS Decellularisation of unscarred and scarred dermis was successfully achieved. The parameters addressed in this study should be carefully considered when developing engineered scaffolds for dermal wound repair. Ideally, the scaffolds should exhibit a mesh-like structure with a rough surface and low stiffness, which represents the microenvironment of unscarred dermal tissue.

N1.04

Dynamic Fibroblast Contractions Attract Remote Macrophages In Fibrillar Collagen Matrix

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Intimate communication between macrophages (Mϕ) and fibroblasts is important for tissue repair after injury and miscommunication can lead to pathological healing and fibrosis. The guidance cues and mechanisms directing migratory Mϕ towards collagen producing and contracting fibroblasts are unknown. We show that contracting fibroblasts generate deformation fields in fibrillar collagen matrix that provide far-reaching physical cues for Mϕ. When positioned within fibroblast deformation fields, Mϕ migrated towards the contraction source from distances of hundreds of micrometers. Mϕ chemotaxis was excluded by eliminating possible cytokine gradients with fluid flow and replacing fibroblasts with actuated microneedles as the force centre. Microneedle experiments identified the presence of a dynamic force source as the critical signal in the matrix to initiate and direct Mϕ migration. In contrast, collagen condensation and fiber alignment resulting from fibroblast remodelling activities were neither required nor sufficient to guide Mϕ migration. We propose a novel mechanism of far-ranging Mϕ mechanosensing that integrates locally sensed displacements of the substrate. We conclude that dynamic fibroblast contractile events are transmitted through fibrillar matrix and critical to attract Mϕ over distances that exceed the range of chemotactic gradients.

N1.05

Isolation And Characterization Of Pericytes From Burn Eschar Tissues

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BACKGROUND: Pericytes, a population of mesenchymal stem cells, that are capable of differentiating into fibroblast/myofibroblasts phenotype promoting fibrosis and scar contractures. We hypothesize that in the burn wound environment, pericytes dissociated from vessels are in a hyperactive state in response to the various factors secreted by activated immune cells. These hyperactive pericytes can become predisposed due to excessive PDGF in the wound environment and differentiate into myofibroblasts promoting excessive fibroplasia. METHODS: To test this hypothesis, we utilized a novel approach to isolate and culture pericytes from patients undergoing burn wound debridement. RESULTS: Discarded burn wound eschar tissues and normal skin tissues obtained from patients undergoing elective plastic surgery procedures were processed without enzyme treatment by gently scraping the tissues into pericytes specific-growth medium. Within 3-4 days, an outgrowth of cells was observed and pericytes were analyzed and sorted directly for $CD146^{+}/CD73^{+}/CD105^{+}/CD56^{-}/CD34^{-}/CD45^{-}$. FACS analyses showed increased percentage of pericytes (~67%) in burn eschar tissues as opposed to normal skin tissues (~21%). FACS sorted pericytes were allowed to grow and expand *in vitro* using pericyte-specific growth medium. Immunofluorescence studies using CD146 and anti-fibroblast antibodies confirmed the presence of pericytes and absence of fibroblasts. Immunohistochemistry revealed increased amount of CD146 positive cells in burn eschar tissues compared to normal skin tissue. RNA-seq studies are in progress to understand the transcriptomic changes of pericytes in burn wound environment and to focus on the genes that contribute to pericyte differentiation (PDGF, TGF-β/Smad 1/2, ADAM12, TWEAK, FN14, CD248 (endosialin), Wnt) to fibroblast/myofibroblasts phenotype. CONCLUSIONS: Results from these studies may provide clues to modify pericytes in the burn wound environment for better healing outcome in reducing scar contractures.

N1.06

Decellularized Keloid Matrix As A Novel Three-Dimensional Model For Studying Cellular Behavior Of Abnormal Keloid Fibroblasts

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BACKGROUND The pathophysiology of keloids involves a complex orchestrated series of processes, which has yet to be explored and fully defined. In particular, the interaction between the various populations of cells within keloids and the keloid matrix is unclear, with many matrix molecules having been implicated in its development as a result of dysregulated homeostasis. Nevertheless, research in keloids is largely hindered by the lack of effective animal models. Despite the use of different materials and animals to model the disease, there is a profound difficulty in exploring the cell-matrix interactions in keloids as a function of the keloid micro-environment. This is important as the role of mechanobiology has been increasingly shown to be crucial in the pathogenesis of many other diseases. METHODS Using tissue engineering methods, we hereby propose a novel model using decellularized scaffolds to study the behavior of keloid fibroblasts in a three-dimensional environment which retains features of these mechanical properties. Punch biopsies obtained from decellularized keloid and normal skin samples were seeded with keloid and normal dermal fibroblasts and subsequently maintained for 14 days. The proliferation rates of keloid fibroblasts in the two groups (n=5 each) were measured and compared at days 3, 7 and 14 using PrestoBlue. RESULTS We found that keloid fibroblasts exhibited significantly higher proliferation rates in the keloid scaffold group. Our results suggest that there may be structural as well as intrinsic properties within the keloid matrix, which are absent in normal skin, driving the accelerated growth of keloid fibroblasts following disease activation. CONCLUSIONS More importantly, we have developed a viable model to study keloid behavior in a three-dimensional environment which closely resemble their original matrix, thereby facilitating further study of cell-matrix interaction in more detail.

WHS SESSION N: Concurrent Session: Chronic Wounds
Saturday, April 28, 2018 9:15 A.M. - 10:15 A.M.

N2.01

Importance Of Oxidative Stress On The Initiation Of Chronic Wound Development In A Diabetic Chronic Wound Mouse Model

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Human chronic wounds have high levels oxidative stress (OS) but to date it is not known how OS contributes to initiation of chronicity. Most of the time well-controlled experiments cannot be performed in humans. However, animal models have proven very effective in understanding disease in humans. We have developed a model of chronic wounds in diabetic mice that mimic many of the aspects of human chronic wounds including biofilm developing naturally, i.e. without the introduction of external bacteria into the wound. To create the chronic wounds in this mouse model, we used inhibitors of anti-oxidant enzymes to increase levels of OS. We hypothesize that oxidative stress levels are critical for development of chronicity including biofilm formation. We used different concentrations of inhibitors of anti-oxidant enzymes to cause OS and found that the wounds healed better as the levels of inhibitors, used to induce OS, decreased. To further determine whether OS influences the quantity and quality of the microbiome, we collected the biofilm over time, analyzed it using bacterial intergenic transcribed spacer sequences and found differences in microbial composition of wounds that heal compared to wounds that remain chronic. To further determine the importance of OS in chronic wound development, we took biofilm from fully chronic wounds and applied it to new wounds without increasing OS. We found that in the absence of OS the biofilm alone was unable make the wounds chronic. Conversely, increasing OS in wounds, when keeping the wounds clean, delayed chronicity, suggesting that both high levels of OS and bacteria are needed for initiation of chronicity. *In conclusion*, we propose that level of OS in a wound may be a good predictor of degree of chronicity and that managing OS in chronic wounds after debridement could lead to wound closure and improved healing preventing return of chronicity.

N2.02

N-acetyl-cysteine Disassembles Bacterial Biofilm And Causes Cell Death Leading To Disappearance Of The Biofilm And Improved Wound Healing

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Chronic ulcers have become a major challenge to healthcare systems worldwide. The presence of biofilm significantly prevents healing of chronic wounds, suggesting the need to prevent biofilm development or, if present, dismantling the biofilm without adversely affecting the host cells. Despite the availability of various treatments for chronic wound biofilm from antibiotics, to antiseptics, to chlorine and oxygen reactive species, none of them are effective in dismantling biofilm and simultaneously killing the bacteria. We have previously shown that *N*-acetyl-cysteine (NAC) significantly improves the healing of biofilm-containing chronic wounds in a diabetic mouse model. NAC dismantles the extracellular polymeric substance (EPS) of the biofilm and leads to disappearance of the bacteria from the wounds resulting in healing. We hypothesize that NAC creates an environment that disrupts phenazine binding to eDNA and/or proteins leading to dismantling of the biofilm and that it lowers the pH causing death of the bacteria embedded in the EPS. To test this hypothesis, we performed studies *in vitro* using biofilm from the chronic wounds of our mouse model containing primarily *Pseudomonas aeruginosa*. When NAC was added to developed biofilm, we found that the biofilm dismantled and the bacteria in it did not grow in rich media. This effect was dose dependent, with the most effective doses of NAC being the ones that create a pH close to or below the NAC pKa. A similar effect was seen when NAC was added before biofilm formation. Staining with pHrodo Red AM intracellular pH indicator showed that the bacteria cytosol stained intensely suggesting that the pH is low and may indicate bacterial cell death. These results suggest that NAC disrupts biofilm structure and bacterial survival by unbalancing the biofilm oxidative state. These findings may provide guidance to develop new antimicrobials and effective treatments of diabetic chronic wounds.

N2.03

Global Gene Dysregulation Due To High Oxidative Stress Leads To Chronic Wound Initiaton

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Wound healing involves a series of sequential processes that are regulated in a temporal and spatial manner leading to successful tissue repair after injury. Chronic wounds develop as a result of defective regulation of one or more of these complex processes, but how these processes are affected during chronicity initiation is still not known. We used a diabetic mouse model of chronic wound to study gene regulation during initiation of chronicity. Cutaneous tissue was collected between 24 hours and 5 days post-wounding from mice treated with vehicle or inhibitors for the antioxidant enzymes, catalase and glutathione peroxidase, to increase the oxidative stress (OS) in the wound and stimulate chronicity. RNAseq was performed and the data processed and analyzed via systempipeR and biomaRt. Within 24 hours, over 2000 genes are differentially expressed in chronic wounds. Most are up-regulated and they include transcription factors from the Fox, Hox, Grh and AP-2 families, chromatin remodeling factors such as SNF/SWI and histone acetyltransferases (HATs). Gene ontology analysis shows that these genes stimulate biological processes such as membrane and cytoskeleton organization, cell death, cell proliferation, differentiation, adhesion, migration, inflammation and angiogenesis, all of which are critical for proper granulation tissue formation and wound closure. In contrast, most genes are significantly downregulated by Day 5, indicating that the wound is in disarray and cannot proceed to proper healing. These results suggest that high OS levels stimulate large-scale transcription activation early in the healing process, leading to turning on processes that should not occur early after injury whereas these processes are not turned on later when they are needed for proper healing. *In conclusion*, understanding the effects of high levels of OS shortly after debridement of human wounds, could lead to the development of treatments that can reverse the course of non-healing to healing.

N2.04

Renal Dysfunction Aggravated Impaired Diabetic Cutaneous Wound Healing

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Background: Renal dysfunction has been associated with an increased incidence of foot ulcers as well as worsened outcomes of wound healing in the diabetic population. The purpose of this study was to create an excisional wound healing model in diabetic mice with renal dysfunction to investigate the combined effects of diabetes and nephropathy on cutaneous ulcers. Methods: Renal impairment was introduced in diabetic *db/db* mice through unilateral nephrectomy and electro-coagulation of the contralateral kidney. Renal function was subsequently monitored with blood urea nitrogen (BUN) assays throughout the study. After 8 weeks, splinted, full thickness excisional wounds were created on the dorsal skin, and harvested on postoperative days (POD) 7 and 14 for further measurement of wound healing parameters including proliferation, angiogenesis, inflammation , reactive oxygen species, and apoptosis through histology, immunostaining and quantitative PCR (qPCR). Results: Renal injury promoted the increase of BUN in three weeks after initial operation, and maintained at a doubled level compared to control throughout the entirety of the study. Diabetic mice with renal injury displayed notably impaired wound healing processes represented by decreased re-epithelialization (Keratin 14) and granulation tissue deposition, concurrent with significant reductions in cellular proliferation (Ki67) and angiogenesis (CD31), as well as significant increases in inflammatory response (M1 macrophages), oxidative stress (nitro-tyrosine) and cellular apoptosis. Furthermore, qPCR results also displayed corresponding changes of related genes (TNF-α, IL-1β, SOD2) in the wounds of renal injured *db/db* mice. Conclusions: Renal manipulation through unilateral nephrectomy with electro-coagulation of the contralateral kidney accelerated the progress of renal impairment, which was demonstrated to aggravate impaired cutaneous wound healing in diabetic mice.

N2.05

The Significance Of Friction And Shear In The Prevention Of Contemporary Hospital Acquired Pressure Ulcers

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BACKGROUND: Hospital acquired pressure ulcers (HAPU) are largely preventable yet still common occurrences in hospitals. Using case control methodology, this study sought to better understand contemporary factors contributing to HAPU development. METHODS: A case control study was performed of HAPUs over an 8-month period at Yale New Haven Hospital (YNHH). A Cox Regression Analysis model analyzed the impact of multiple factors on HAPU development including friction and shear, among other Braden score components. A Receiver Operating Characteristic (ROC) curve was calculated to determine the sensitivity and specificity of changes in these factors in predicting HAPU development. RESULTS: On a sample of 9,145 admissions, HAPU incidence was 4.4% over the study period (6.6% per annum). The average hospital day for HAPU development was day 14.0 (± 19.0). The Cox Regression Analysis demonstrated that the friction and shear component of Braden scores had a hazard ratio of 26.81 (p-value<0.01, CI: 15.49-46.40), meaning an increase of 1.0 in the standard deviation of the friction and shear component was associated with 26.81 fold increase in HAPU risk. Change in the friction and shear component was the most predictive factor with a high ROC curve area of 0.851±0.01 (CI: 0.833-0.869). CONCLUSION: Change in the friction and shear component of Braden scores appears to be the most significant factor preceding HAPU development at YNHH. Efforts to place more focus on preventing changes to this factor may help decrease HAPU risk for future patients.

N2.06

Effects Of Noncontact Low Frequencyultrasound (nlfu) On Wound Healing At The Molecular Level

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Background: Chronic-venous-leg-ulcer (CVLU) healing-rates are less than 70% with standard care resulting in rising costs. New therapies are needed to increase healing-rates and reduce healing times. NLFU* is used to treat various types of chronic wounds including venous, diabetic and pressure ulcers. Aim: Objective for this sub-study of the IN-BALANCE-RCT-VLU-trial was to characterize and compare NLFU*-treatment-group and patients receiving standard-of-care (SOC) for effect of treatment on content/quantity of inflammatory cytokines, fibrinogen and bacteria. Methods: 36 subjects with CVLUs were randomized to receive NLFU*-plus-SOC or SOC-alone. Wound-area-reduction was evaluated weekly. PF4, TGF-beta, and fibrinogen were identified using immunohistochemistry. IL-1beta, TNF-alpha, IL-6, IL-8, and IL-10 were measured by multiplex-immunoassay. PathoGenius for 16S-rRNA-marker-gene-tag-sequencing and qPCRs was used to assess bacteria. Results: Higher wound-area-reduction was observed in NLFU*-group (67.0%) compared to SOC-group (41.6%, $p<0.05$). Anaerococcus, Peptoniphilus, and Finegoldia had the highest median proportion in samples overall. Bacterial load determined local parameters of ulcer inflammation. Peptoniphilus abundance decreased more with NLFU*-treatment relative to SOC; similar trends were observed for Anaerococcus and Finegoldia. Fibrinogen amounts significantly diminished over time by NLFU*-treatment ($p<0.05$) and IL-8 levels declined. Conclusions: NLFU*-treatment is an effective adjuvant tool for CVLU therapy. Data at cellular level demonstrated that NLFU* improves wound healing by equally inhibiting abundant levels of pro-inflammatory cytokines and reducing overall bacterial burden. *MIST-Therapy-System5.0®, Alliqua™BioMedical

WHS SESSION N: Concurrent Session: Inflammation and Immunity
Saturday, April 28, 2018 9:15 A.M. - 10:15 A.M.

N3.01

Chronic Wound Microbiome Colonization on Mouse Model Following Cryogenic Preservation

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It was recently shown that slough isolated from chronic wounds could re-establish polymicrobial biofilm infections in the mouse model, providing an experimental design to study patient wound biofilm using an *in vivo* model. However, there are practical limitations of sample collection, timing and transportation for wounding procedures that complicate such experiments. The purpose of this study was to investigate cryogenic preservation on the ability of polymicrobial biofilms to re-establish in a mouse wound model. Slough from five patients was homogenized and divided into three preservation strategies which included refrigeration until infection as previously reported, being frozen in liquid nitrogen, or being placed in glycerol solution before freezing in liquid nitrogen. Individual mice were subsequently infected with slough treatments and were matched with controls. Four days following inoculation, wound microbiota were characterized by 16s rDNA community profiling and bacterial load by quantitative PCR. Analyses were conducted to understand the effect of patient origin and preservation strategy on microbiome community composition. It was found that patient origin explained a significantly greater amount of variation than treatment, which indicated original patient wound microbiome could be partially re-established in a mouse model following preservation. The prior relative abundances of individual species in slough from patients had a significant positive relationship with colonization success in mice. Wound microbiome diversity was also found to be negatively associated with bacterial load, indicating a relationship between microbiome community diversity and bioburden. Cell viability comparisons among preservation treatments were also made with samples from an additional 11 patients where it was found that freezing did not present a significant reduction in viability. Although it was known that metagenomic studies can be enhanced by cryogenic archives, results of the current study indicate an expanded utility in biofilm and microbiome research.

N3.02

Collagenase Resolves Wound Inflammation Through A Pge_2-ep4-stat6 Mediated Pro-healing Macrophage Polarization

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Background- Debridement is a necessary component of bed preparation in wound care. Clostridial collagenase, marketed as Collagenase Santyl Ointment (CSO), is FDA approved for such use. Building on the scientific premise that collagenases and collagen degradation products may regulate immune cell function, we sought to investigate the potential role of CSO in regulating wound inflammation. We tested the hypothesis that in addition to enacting debridement, CSO contributes to the resolution of persistent wound inflammation. Methods- Wound macrophages were isolated from PVA sponges previously loaded with CSO or petrolatum (control) and implanted subcutaneously on the back of male mice. Results- CSO treatment significantly increased pro-healing ($m\phi^{heal}$) and decreased pro-inflammatory ($m\phi^{inf}$) polarization in acute as well as diabetic wounds macrophages ($p<0.05$; $n=5$). CSO-treated wound macrophages functionally displayed increased production of anti-inflammatory cytokines IL-10 and TGF-β, but decreased pro-inflammatory cytokines TNF-α and IL-1β ($p<0.05$; $n=5$). The active ingredient of CSO, CS-API, induced the expression of $m\phi^{heal}$ and downregulated $m\phi^{inf}$ polarization markers *ex vivo* ($p<0.05$; $n=4$). Murine RAW 264.7 macrophages were used to identify the transcriptional regulation of CS-API mediated $m\phi^{heal}$ polarization. CS-API treatment attenuated transactivation of pro-inflammatory transcription factor NF-κB, and induced the activity of STAT6 ($p<0.05$; $n=5$), a transcription factor involved in macrophage $m\phi^{heal}$ polarization. Inhibition of STAT6 significantly abrogated the anti-inflammatory effects of CS-API ($p<0.05$; $n=4$). PGE_2 drives $m\phi^{heal}$ macrophage polarization through EP4 receptor. CS-API treatment significantly increased PGE_2 in macrophages ($p<0.05$; $n=3$). Blocking the EP4 receptor significantly attenuated the CS-API-induced phosphorylation of STAT6 and decreased the shift towards $m\phi^{heal}$ phenotype ($p<0.05$; $n=3$). Conclusion- This work presents first evidence demonstrating that CSO, otherwise known as a debridement agent in wound clinics, is powerful in resolving wound inflammation *via* PGE_2-EP4-STAT6 pathway.

N3.03

Granzyme B In Sub-epidermal Blistering

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INTRODUCTION: In healthy skin, the epidermis and dermis are anchored together at the dermal-epidermal junction (DEJ), a specialized basement membrane pivotal for skin integrity and function. However, in sub-epidermal bullous conditions, the DEJ is compromised resulting in DEJ disruption and separation resulting in blistering. Although the etiology of these conditions can vary, they all involve epidermal detachment leading to increased risk of infection and reduced quality of life. Granzyme B (GzmB) is a pro-apoptotic serine protease secreted by immune cells that can cleave extracellular matrix proteins. Although previous studies have observed abundant levels of GzmB in the DEJ, a non-apoptotic role in blistering has never been considered. The present study suggests that GzmB cleaves key DEJ proteins leading to epidermal detachment and blistering.

METHODS: Cleavage assays and TAILS analysis were performed to identify and confirm novel GzmB substrates in skin. Collagen VII, α6β4 integrin and Collagen XVII were identified as substrates. To investigate whether the aforementioned substrates are cleaved in vivo, immunostaining for GzmB and ECM substrates were performed on sub-epidermal blistering diseases (Bullous pemphigoid, dermatitis herpetiformis, SJS/TEN, and epidermolysis bullosa). Finally, sections of human skin were exposed to GzmB to investigate whether GzmB could induce DEJ disruption.

RESULTS: Cleavage of ECM substrates was confirmed by western blotting and ATOMS was utilized to identify cleavage sites. Ex vivo studies indicated that GzmB could induce DEJ separation; a process that was inhibited by GzmB inhibition. Excitingly, all sub-epidermal blistering conditions exhibited increased GzmB and reduced Collagen VII, alpha6beta4 integrin and Collagen XVII specifically in the area proximal to DEJ disruption and blistering.

CONCLUSIONS: GzmB may be a common causative link in sub-epidermal blistering and thus could be used as a broad approach to preventing blistering in such conditions.

N3.04

Hyperglycemia Induces Long Non-coding Rna Gas5 Expression Through The Ribosomal Binding Protein Hur

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Background: Diabetic wounds exhibit prolonged accumulation of macrophage with elevated levels of proinflammatory cytokines. We have previously shown that lncRNA GAS5 (Growth Arrest-Specific 5) was up-regulated in diabetic wounds, and the persistence of the proinflammatory macrophage (M1) phenotype was mediated partly by GAS5/STAT1 pathway, indicating a potential role for GAS5 in the pathogenesis of diabetic wounds. RNA binding protein HuR stabilizes mRNA by binding to 3'UTR of mRNA and inhibiting microRNA binding. We hypothesize that hyperglycemia induces GAS5 expression by alteration of HuR binding. Methods: To test our hypothesis, we incubated the murine macrophage cell line RAW264.7 with media containing 5 mM glucose (low glucose), or 25 mM glucose (high glucose) for 24 hours. RNA immunoprecipitation (RIP) was used to analyze HuR binding and Real-time PCR used to quantify relative gene expression. Results: GAS5 was significantly up-regulated in high glucose conditions. Under low glucose conditions, the level of GAS5 was not significantly different between the anti-HuR group and IgG control group. Under high glucose conditions, the level of GAS5 in the anti-HuR immunoprecipitated lysate was significantly higher than in the IgG control group. Discussion: These findings demonstrate a novel mechanism in the regulation of the effects of lncRNA GAS5 expression, with HuR binding to lncRNA GAS5 and hyperglycemia induces HuR binding to GAS5 to stabilize GAS5. Furthermore, these results may represent a potential novel therapeutic target to correct the impaired diabetic wound healing response.

N3.05

Investigation Of Endogenous Gene Expression Changes After Vegf Gene Therapy Via Aav2 Double-stranded Vectors

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Rhode Island Hospital, Providence, RI, USA

Background: We previously reported that diabetic mouse (db/db) wound healing is significantly delayed with downregulated gene expression of multiple growth factors and their receptors including bFGF, PDGFb and VEGF. In this study, we aimed to investigate if adeno-associated double-stranded viral vector 2 (AAV2-ds) mediated VEGF gene therapy activates endogenous wound healing related genes. Methods: In 12 db/db mice, dorsal paired 8 mm-diameter wounds were created. 10^{11}viral particles of AAV2-ds-VEGF or AAV2-ds-GFP diluted in saline were injected intradermally into the wound edge and the wound bed, 6 mice per group. At day 15 and 21 post-wounding, six wounds from each group were formalin-fixed, paraffin-embedded (FFPE) and sectioned. Immunohistochemistry was used to verify the success of VEGF gene transfer. Total RNA was extracted from FFPE sections, wounds and edges separated. RT-qPCR was performed to examine gene expression of bFGF, EGF, IGF1, FN, MMP9, PDGFb, TGFb1, TIMP1, and VEGFa. Results: We verified significantly increased VEGF staining in AAV2-ds-VEGF treated wound granulation tissue at day 15. At day 15, the expression of PDGFb and TIMP1 genes was significantly increased ($p<0.05$ and $p<0.01$) in AAV2-ds-VEGF group compared to that in AAV2-ds-GFP group; the EGF gene expression was significantly higher in AAV2-ds-VEGF treated wounds than that in AAV2-ds-GFP treated wounds. At day 21, the expression of EGF gene in AAV2-ds-VEGF group was significantly decreased than that at day 15 ($p<0.05$). There was no significant difference in other studied genes. Conclusions: VEGF gene was successfully transduced by AAV2-ds vectors. The exogenous VEGF gene significantly increased endogenous genes specific to wound healing after AAV2-ds-VEGF therapy. Further optimization of AAV2-ds vector mediated VEGF gene therapy could improve the diabetic wound healing.

N3.06

An Effective "Anti-Inflammatory/Anti-ROS" Combination Therapy That Accelerates Diabetic Wound Healing

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Background: Diabetic wounds have become one of the most challenging public health issues of the 21st century with an annual cost of $1.5 billion in the US alone and yet no effective treatment is available. Diabetic wounds don't heal properly due to many deficiencies including elevated inflammation and oxidative stress. We have previously shown that this abnormal inflammatory response is due to decreased levels of a key regulator of the NFkB pro-inflammatory signaling, miR-146a. We have also shown that cerium oxide nanoparticles (CNP) act as antioxidants and scavenge reactive oxygen species (ROS) in wounds. Thus, we propose the hypothesis that combined targeting of inflammation and oxidative stress via local delivery of CNP carrying miR-146a (CNP+miR-146a) will effectively improve diabetic wound healing. Methods: 8 mm full-thickness wounds were created on the dorsal skin of diabetic and non-diabetic mice and treated with 10^8pfu Lenti-miR-GFP, 10^8pfu Lenti-miR-146a, or 10uM of CNP+miR-146a. Rate of wound closure was measured until the wounds were fully healed. Subsets of these wounds were harvested 7 days post-wounding and miR-146a, NFkB, IRAK1, TRAF6, IL-6, IL-8, and NOX2 gene expression was analyzed. Inflammatory cell infiltration was analyzed by CD45 immunostaining. Results: CNP+miR-146a effectively decreased the: activation of NFkB pro-inflammatory signaling, upregulation of IL-6 and IL-8 levels, recruitment of pro-inflammatory CD45+ cells, and NOX2 (ROS producer) expression. These corrections lead to a dramatic improvement in the rate of diabetic wounds closure. Conversely, although it was successful in decreasing inflammation, Lenti-miR-146a did not accelerate diabetic wound closure. Conclusions: Our findings demonstrate that "CNP+miR-146a Combination Therapy" significantly accelerated diabetic wound healing by modulating inflammation and oxidative stress. This novel therapy has the potential for future clinical application and could bring new hope to patients suffering from diabetic wounds.

N4.01

Next Generation Sequencing Reveals Novel Mechanism Of Statin Action To Promote Healing In Pre-clinical And Clinical Models

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Statins, HMG-CoA reductase inhibitors, primarily used as cholesterol reducing agents, represent a promising new therapeutic modality for treatment of non-healing wounds. We show that statins promote epithelialization *in vivo* and *ex vivo* using porcine and human *ex vivo* wound models. To reveal molecular mechanisms of action we performed next generation sequencing of mevastatin-treated primary human keratinocytes. RNA-seq analysis followed by functional confirmation revealed that mevastatin induced cell migration while inhibited cell proliferation, suggesting that statins may shift the chronic wounds from a hyper-proliferative to a migratory phenotype to promote healing. Ingenuity pathway analyses identified the epidermal growth factor receptor (EGFR) signaling as the major pathway modulated by mevastatin. We confirmed that mevastatin blocked keratinocyte proliferation by inhibiting expression of cell cycle genes while promoting EGF-induced keratinocyte migration. More importantly, we found mevastatin restored EGF signaling cascade in diabetic foot ulcer tissue, suggesting statins can re-sensitize patients to EGF stimulation. Furthermore, we found that mevastatin inhibited cortisol synthesis, a potent inhibitor of keratinocyte migration and epithelialization, and its downstream target c-myc, a biomarker for non-healing wound tissue and major activator of hyper-proliferation. This inhibition occurred through statin-induced expression of the long non-coding RNA, Gas5. We conclude that mevastatin promotes wound healing through multiple complex mechanisms including restoring EGFR and suppressing cortisol pathway, and through regulating molecular activators and inhibitors of wound healing to facilitate therapeutic reprogramming.

N4.02

PEG-Plasma Hydrogels Increase Epithelialization Using A Human Ex Vivo Skin Model

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Background: Ex vivo wound healing models are an alternative to in vitro cell culture and are better at investigating proliferation, differentiation, and migration of cells in their natural three dimensional environment. The purpose of this study was to establish an ex vivo model from discarded human skin to evaluate therapies aimed at improving wound healing. Methods: An 8-10 mm biopsy “wound” was created in the center of a 6-well insert sized piece of discarded skin and incubated with media maintained at the epidermal/dermal border to keep the epidermis air exposed. Three hydrogels [collagen, polyethylene glycol (PEG)-fibrin, or PEG-plasma] were tested in the wounds. Microscopy images were captured to measure the epithelialization. After 14 days, the tissue was fixed and stained for cytokeratin 10 (CK-10), alpha smooth muscle actin (α-SMA), and wheat germ (WG). Results: Collagen treated wounds resulted in minimal cellular proliferation and migration. The PEG-plasma hydrogel treated wounds epithelialized faster than other groups at days 8, 11, and 14 (76.8, 90.7, 97.4 vs. 34.1, 41.6, 54.9%, $p<0.01$, respectively vs. PEG-fibrin). Sections co-stained with WG and α-SMA indicated cells from the normal tissue had infiltrated and proliferated into the PEGylated hydrogels. On top of the PEG-plasma hydrogels, a 5-7 cell layer thick CK-10 positive stratified epidermis was observed. Conclusions: The PEG-plasma hydrogels allowed the wounds to epithelialize with a stratified epidermis at a faster rate than wounds treated with other biomaterials. Development of a practical ex vivo skin model is superior to other standard in vitro cell culture and can be used as a screening tool to study wound healing to minimize the number of animals used in research.

N4.03

Platelet Rich Plasma Treatment Accelerates Re-epithelialization In A Murine Model Of Excisional Wound Healing

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Background: Rapid wound closure is critical, as intact skin is the body's barrier against insult by environmental factors. Therefore, it is of interest to develop a safe and effective means for accelerating wound healing. Platelet-Rich Plasma (PRP) when activated, releases growth factorsthat may contribute to accelerated wound healing. Methods: PRP was created by multiple rounds of centrifugation of citrated whole blood. Punch biopsies (6mm) were used to create two wounds on the dorsum of C57BL/6 mice. Splints were placed on the wounds to encourage healing by re-epithelialization instead of contraction. One group of animals received no treatment, while another received PRP. PRP was activated by $CaCl_2$ and recombinant thrombin to form a gel that was applied to each wound. Photos of wounds were taken prior to necropsy at days 3, 5, or 7 post-injury and entire wounds were fixed in formalin. Open wound areas were quantified using Image J to assess rates of healing. The fixed wounds were stained with H&E or Cytokeratin 16 and were examined histomorphometrically for re-epithelialization. Results: Wright giemsa staining confirmed platelet concentration in PRP compared to whole blood. PRP-treated wounds re-epithelialized faster compared to untreated wounds when wound photos were analyzed at Days 3 and 5 ($n=6$ wounds, $p<0.05$). Open wound areas were smaller in treated wounds when H&E and Cytokeratin 16 sections were analyzed. Conclusions: Due to its autologous nature, PRP serves as a safe and efficacious option for accelerating wound healing.

N4.04

Skin-specific Hyaluronan Knockdown In Mice By An Optimized Topical 4-methylumbelliferone Formulation

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Background: Hyaluronan (HA) is prominently abundant in the skin; while HA can be synthesized by the HAS1-3 enzyme family, HAS2 is the leading contributor. Dysregulation and accumulation of HA is implicated in the pathogenesis of several diseases such as keloid scarring and metastatic melanoma. To understand how HA expression contributes to the development of fibrotic disorders, we propose the development of a skin-specific HA knockdown model, which tests an optimal delivery system of topical 4-methylumbelliferone (4MU). Methods: Skin HA content was measured in male and female mice (n=30, M/F) at 1,4,7,14, and 24w. A design-of-experiments(DOE) approach was employed to develop an optimal 4MU skin-delivery formulation comprising a combination of propylene glycol(PG), ethanol(EtOH), and water(n=40; 7w M/F). This was topically applied twice daily for 7days to compare HA knockdown levels between topical 4MU, topical control, 4MU chow, and diet control (n=24; 6w M/F). Serum and skin samples were harvested to analyze HA content (HA-ELISA, immunohistochemistry) and HAS1-3 expression (qRT-PCR). Average+/-SD; $p<0.05$ were assessed by ANOVA. Results: HA content in dorsal (344.3+/-67.2ng/mg of skin) and ventral (324.7+/-46.8ng/mg) skin was comparable at all ages and between sexes. Consistent with our prediction of 70% knockdown, the optimal formulation of 0.82 mM 4MU in PG(16.32%;v/v)+EtOH(15.71%;v/v)+water(67.97%;v/v) resulted in 60% reduction of HA in dorsal skin($p<0.05$), with 68% HA knockdown($p<0.01$) in female mice compared to 22%($p<0.05$) in males. 4MU topical application resulted in a significant decrease in dermal HAS2 expression (3-fold M+F;$p<0.05$). No significant effect of topical 4MU was observed in HA serum levels compared to controls. Histologic analyses showed thicker dermis in 7w male mice, whereas female mice had a predominant adipose layer; topical 4MU resulted in a significant breakdown in HA expression pattern. Conclusions: Our data suggest a 4MU formulation model that can be invaluable in elucidating the sex-specific and skin-specific effects of hyaluronan in normal and pathologic states of wound healing.

N4.05

Substance P Promotes Fibrosis In Human Corneal Stroma

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Substance P (SP) is a neuropeptide which has been shown to be present in human corneal cells, keratocytes. Many studies suggest its role in various cellular processes important in wound healing such as proliferation or migration. We hypothesize that SP regulates expression of keratocyte markers, extracellular matrix (ECM) components and fibrotic markers that are overexpressed during fibrosis, in both primary keratocytes and myofibroblasts. Primary keratocytes, which were isolated from healthy human corneas obtained from the local cornea bank, and an *in vitro* corneal fibrosis model (myofibroblasts) were used throughout this study. The effect of SP on keratocyte and myofibroblast contractile abilities was assessed by cell contraction assay. Gene expression of keratocyte markers (keratocan and aldehyde dehydrogenase 3 family, member A1 [ALDH3A1]), ECM components (collagen I, collagen III, collagen V and lumican), and markers of fibrosis (α-smooth muscle actin [α-SMA] and fibronectin), was determined by qRT-PCR. Treatment of keratocytes with SP resulted in decreased expression of keratocan gene but increased ALDH3A expression. SP increased expression of fibrotic markers, α-SMA and fibronectin. Moreover, collagen I, collagen III and collagen V genes were also upregulated by SP. Expression of lumican was unaffected by SP. Furthermore, keratocytes treated with SP showed increased contractile abilities. Similar effects of SP were observed in the corneal fibrosis model. SP decreased keratocan, but increased ALDH3A1 gene expression. α-SMA, fibronectin, collagen I, collagen III and collagen V genes were upregulated. Expression of lumican was unaffected. Contractile abilities of myofibroblasts increased upon SP treatment. In conclusion, SP is able to regulate keratocyte marker genes and to increase expression of various ECM genes and fibrotic markers in both keratocytes and myofibroblasts. This suggests that SP might promote fibrosis in human cornea.

N4.06

Omega-3 Rich Fish Skin Grafts Reduce Donor Skin Requirements For Full Thickness Burns

Randolph Stone, II [1], David Larson[1], John Wall[1], Kyle Florell[1], Hannah Dillon[1], Skuli Magnusson[2], Hilmar Kjartansson[2], Shanmugasundaram Natesan[1], Robert Christy[1]

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Background: Allografts (cadaver skin) are routinely used for treatment of burn injuries as a temporary covering to protect the wound while donor sites heal. However, allografts aren't always available and have high cost associated with them. The purpose of this study was to evaluate fish skin graft (FSG) as a temporary cover to prepare the wound bed for meshed split thickness skin graft (mSTSG) application and as protection over a highly mSTSG. Methods: Full-thickness 5x5 cm burn wounds were created on the dorsum of anesthetized Yorkshire pigs using appropriate pain control methods. Twenty-four hours post-burn, day 0 (D0) wounds were excised down to a viable wound bed and a temporary cover was applied. Then on day 7 (D7), wounds were grafted with a mSTSG. Thirty six wounds were divided into three groups: 1) (D0) FSG then (D7) 1.5:1 mSTSG; 2) (D0) cadaver skin then (D7) 1.5:1 mSTSG; 3) (D0) FSG then (D7) 3:1 mSTSG and FSG applied over the graft. Quantitative measurements include contraction rates, transepidermal water loss (TEWL), hydration, and blood flow. Results: Wounds treated with FSG had similar quantitative measurement outcomes compared to cadaver skin treated burn wounds. The 3:1 mSTSG applied with FSG resulted in similar healing as the wounds treated with the 1.5:1 mSTSG. Conclusions: FSG was found to be non-inferior compared to cadaver skin as a temporary cover as both resulted in similar healing. Most importantly, the wounds treated with FSG and 3:1 mSTSG required 50% less graft and resulted in no meshed pattern typically observed with highly meshed grafts.

WHS POSTER SESSION
Friday April 27, 2017 7:15 pm – 8:45 pm

(Poster Hall is Open Friday April 27th & Saturday April 28th, 7:30 AM – 6:00 PM)

ACUTE WOUNDS

P.AW01

Acute Wound Healing In A Rodent Model Of Uremia

Sai Krishna Duraisingham, Julius E. Kieswich, Steven M. Harwood, Muhammad M. Yaqoob
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BACKGROUND. Patients with chronic kidney disease develop a multitude of skin changes associated with their renal failure. Causative comorbidities such as peripheral vascular disease and diabetes directly impact healing. Poor healing contributes to prolonged hospital stays, susceptibility to infective complications and significant morbidity, including considerable negative psychological impact. Methods to assess wound healing in uremia will be valuable. METHODS. We developed a rodent model of excisional wound healing. Six week old male Wistar rats were fed a standard diet supplemented with 0.75% adenine for 3 weeks to establish uremia. Healthy controls were fed standard chow. Survival, growth rate and well-being were monitored. Bilateral 5mm full thickness dorsal punch biopsies were made. At day 3 and 7 measurements of the wounds were taken. Blood samples obtained by cardiac puncture were centrifuged for plasma and organs were harvested for analysis. Wounds were excised and bisected, one semicircle section was stored in formalin, the other snap frozen under liquid nitrogen. Experiments were conducted under our UK Home Office license after institutional approval. RESULTS. This model successfully established a uremic state with no premature deaths, excess bleeding or infected wounds observed. Serum urea was significantly higher in the uremic group at both day 3 and 7 (p<0.01) as was serum creatinine (p<0.02). Percentage of the wound area healed compared to day 0 was significantly greater in the control group at day 3 and day 7 (p<0.02 and p<0.001). Sample size n= 5 per group.
CONCLUSIONS. We developed a rodent model with low complication rates to study factors contributing to delayed wound healing in uremia. Moreover, we have demonstrated a delay in healing in uraemia.

P.AW02

Ex-vivo Wound Model To Measure Microbial Burden, Epithelial Toxicity And Biomarker Concentrations Associated With Wound-healing Products

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Background: Although wound models have been described, most in vitro or in vivo models do not reflect conditions in full-thickness human skin. Our aim was to develop an ex vivo human skin model to characterize products for promotion of wound healing (re-epithelialization). Our three-pronged approach employed assays for antimicrobial efficacy, cell toxicity, and effects on biomarkers associated with wound healing. To determine the utility of our model, we investigated Protosan wound gel in comparison to a series of proprietary formulations. Methods: Our model comprised 5-mm explants of full-thickness ex vivo human skin with 2- to 3-mm punch "wounds" that extended into the dermis. For antimicrobial studies: wounds were inoculated with methicillin-resistant *Staphylococcus aureus, Pseudomonas aeruginosa,* or *Acetinobacter baumannii*, and incubated at 37° for 2 h (simulate wound contamination) or up to 3 d to achieve biofilm. After incubation, explants (*n*=3 per treatment) were untreated or treated with Prontosan wound gel or proprietary formulations. For wound healing studies: wounds were untreated or treated with keratinocyte growth factor (KGF, positive control for re-epithelization), Prontosan wound gel or proprietary formulations. Following treatment, explants were incubated for 24h followed by (1) microbial enumeration, (2) cytotoxicity testing (MTT assay), or (3) biomarker concentrations (Human XL Cytokine Discovery Luminex® High Performance Assay, R&D Systems). Results: We identified solutions with optimal wound-healing efficacy, determined by cytotoxicity (≥50% viability) and antibiofilm efficacy (≥2-log reduction in microbial burden compared to untreated explants). We further believe that these formulations are associated with changes in concentrations of wound-healing biomarkers predictive of re-epithelialization. Conclusion: Our model identified biofilm-preventing/wound-healing formulations associated with low cytotoxicity, reduced microbial burden, and changes in concentrations of known wound-healing biomarkers. Our wound-healing model may obviate animal testing and optimize product testing, with consequent faster progression to clinical trials involving acute and chronic wounds in patients.

P.AW03

First Identification Of Volatile Biomarker Profiles And Their Associations In Acute Wound Healing Processes In Human Skin

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BACKGROUND - Volatile organic compounds (VOCs) emanate from human skin and their identification as biochemical markers in the process of wound healing is currently understudied. Therefore, the aim of this novel study was to identify the VOC profiles during the acute phase of cutaneous wound healing.

METHODS - Six healthy male subjects had four 5-mm diameter skin biopsies to their arms. VOC samples were obtained non-invasively from wounded and healthy skin as well as the ambient background at days 0, 7, 14, 21 and 28 using polydimethylsilicone membranes and underwent thermal desorption and were then separated by gas chromatography and detected by mass spectrometry. VOCs were tentatively identified using the National Institute of Standards and Technology library and relative abundances compared. Spectrophotometric intracutaneous analysis, full-field laser perfusion imaging, colorimetry and dynamic optical coherence tomography provided quantitative measurements of melanin, haemoglobin, collagen and blood flow.

RESULTS - Generalised estimating equation model showed significant differences in VOC profiles ($P<0.05$), obtained from wounded skin, healthy skin and background, followed a Gaussian distribution over time (D0-77;D7-150;D14-170;D21-151;D28-112 VOCs). Twenty-five VOCs allowed differentiation between sampling areas independent of sampling time point ($P<0.05$). Twenty-seven VOCs allowed discrimination between wounded from healthy skin at different time points ($P<0.05$). Two-hundred-and-nineteen VOCs significantly varied in wounded skin over the 28 day period ($P<0.05$) of which 41 showed no significant variation in healthy skin which allowed differentiation. Undecane, 3-carene, 1,3-dimethyl-benzene, acetoacetic-3(10)-caren-4-ol, 3,7-bicyclo[4.1.0]heptan-3-ol and 1-undecanol significantly correlated with blood flow ($R>0.7;P<0.001$). Hept-3-yl ester benzoic acid significantly correlated with melanin ($R<-0.7;P<0.001$) and also correlated consistently from D7-28 with both melanin and collagen when comparing across time points ($R<-0.7;P>0.05$).

CONCLUSIONS - For the first time, VOC profiles of the acute phases of wound healing of human skin as potential biomarkers have been identified. Their relationship to the metabolomic and microbiome profile of wounds is currently underway.

P.AW04

Comparison Of Betafoam®, Allevyn®, And Petrolatum Gauze (pg) For Split-thickness Skin Graft (stsg) Donor-site Dressing

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BACKGROUND: Moist wound dressings support rapid healing but non-moist dressings are still commonly used. We evaluated efficacy and safety of a 3% povidone-iodine-containing polyurethane dressing (Betafoam®) for donor-site dressing, versus Allevyn® and PG.

METHODS: This prospective Phase 4 study (NCT02543034) was conducted between Mar-2016 and Apr-2017 at 8 sites in Korea. Consenting subjects (aged ≥19 years, scheduled for STSG) were randomized 1:1:1 to Betafoam®, Allevyn®, or PG dressings for up to 28 days after donor-site collection. We assessed time to complete epithelialization (CE), proportion with CE at Day 14, wound infection, inflammation, pain, treatment acceptability, modified Vancouver Scar Scale (mVSS) score at Day 28.

RESULTS: 98 subjects provided evaluable data (n=31 Betafoam®; n=33 Allevyn®; n=34 PG). Epithelialization time was shortest with Betafoam® (12.74±3.51 days), versus Allevyn® (16.61±4.45 days; p=0.0003, t-test), PG (15.06±4.26 days, p=0.0205). At Day 14, 83.87% of Betafoam® donor-sites had CE, versus 36.36% of Allevyn® donor-sites (p=0.0001, t-test), and 55.88% of PG donor-sites (p=0.0146). There were no wound infections. At Day 28, mVSS scores were 1.47±1.71 (Betafoam®), 2.71±0.37 (Allevyn®), and 3.61±3.54 (PG). Other measures did not differ significantly between groups. Adverse event (AE) incidence was comparable in Betafoam® (17.65%), Allevyn® (37.14%), and PG (29.41%) groups ($\chi 2$ p=0.1940), with no serious AEs; and no dressing-related or skin-related AEs with Betafoam®.

CONCLUSIONS: Betafoam® required less time to complete epithelialization and had a good safety profile.

DISCLOSURE: Abstract submitted in parallel to European Wound Management Association (EWMA) 2018 meeting

P.AW05

Short-term Administration Of A High-fat Diet Impairs Wound Repair In Mice

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Prolonged intake of high-fat diet leads to low grade chronic inflammation and plays an important role in obesity development. Previous studies showed that the long-term administration of a high-fat diet to rats delays cutaneous healing in both obesity-prone and obesity-resistant animals; suggesting that the diet composition was more important in promoting the delay of wound healing than the adipose tissue accumulation. It is also known that short-term administration of high-fat diet was capable of inducing pro-inflammatory pathways in heart, adipose and muscle tissues. However, the effects of a short-term administration of a high-fat diet on skin wound repair are not known. The aim of this study was to investigate the effects of short-term administration of a high-fat diet on cutaneous healing in mice. Mice (n=20) were divided into standard chow (10% of calories from fat) and high-fat chow (60% of calories from fat) groups. After 10 days of diet administration, an excisional lesion was performed and lesion was collected 10 days later. Neither average body weight, nor glucose metabolism were different in studied groups. The high fat chow group presented delayed wound contraction and increased cellular density ($p<0.05$) The high-fat chow group also presented increased neutrophils and macrophages 10 days after wounding ($p<0.05$). The high-fat diet group presented lower type I collagen protein expression; however, 10 days after wounding there was an increase in type III collagen protein expression ($p<0.05$). In conclusion, short-term administration of high-fat diet exerts negative effect on mice cutaneous healing, due to impairment of wound contraction and extracellular matrix deposition and prolongation of the inflammatory phase.

P.AW06

Use Of Abra Dynamic Tissue System And Acell Matristem For Successful Closure Of Traumatic Complex Extremity And Trunk Soft Tissue Wounds

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Background:Traumatic soft tissue injury with tissue loss is a frequent and challenging problem, requiring operations that have long-term functional and cosmetic consequences. This, combined with painful dressing changes, prolonged wound healing, and increased resource utilization, prompted exploration of more effective solutions. ABRA dynamic tissue system (DTS) has been successfully used to facilitate open abdomen closure. There are both invasive and non-invasive variations of the device for closing soft tissue extremity and trunk defects. ACeLL Matristem is a porcine urinary bladder matrix (PUBM) which accelerates wound healing through constructive remodeling.

Objective:We describe a series of patients with combined mechanical and biological closure of complex extremity and trunk soft tissue wounds.

Methods:We identified 14 patients with large complex traumatic wounds and used DTS and PUBM to definitively heal these wounds. In most cases we used both DTS and PUBM and in select patients we used only PUBM. Detailed photographic documentation was performed of each wound.

Results:There was 100% healing of each wound without the need for skin grafting or tissue flaps. There were no surgical site infections (SSI) even in the most contaminated of wounds. In each case, wound healing was accelerated with excellent cosmetic and functional outcomes.

Conclusion:Complex traumatic wounds of the trunk and extremities are a challenging problem. Combined use of DTS and PUBM can be used to successfully heal these wounds.

P.AW07

Intracellular ATP Delivery Induces Enhanced Wound Healing Via Early Initiation Of The Wound Healing Cascade

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BACKGROUND-Intracellular ATP delivery (ATP-vesicles) enhanced wound healing by reducing the traditional 3-6-day lag time, a phenomenon never seen or reported in the past, but the mechanisms are unclear. This study was designed to explore the cellular mechanism in the rapid tissue regeneration.

METHODS-Seventy rabbits were used and four wounds were created on each ear. Two were treated with ATP-vesicles (10 mM Mg-ATP) and the other two were treated with controls (normal saline or Regranex^{TM}). They were sacrificed at 5h, 12h, and days 1, 2, 3, 4, 6, 9, and 15 post-surgery.

RESULTS-Immunohistochemical analysis of the ATP-vesicle-treated wounds showed increase of platelets, neutrophils and cytokines as early as 5-12h by CD61, neutrophil, MCP-1 and TNF-alpha antibodies. Massive macrophage accumulation detected by CD68, CD16 and Anti-Mac by 24h. Stem cells were detected using CD106, CD146 and CD44 antibodies. CD36, arginase and collagen type 1 immunoreactivity were detected as early as day two. Early neovascularization was also detected using CD105, CD34 and CD31 antibodies. Double staining with M1 and M2 macrophage markers showed massive early M2 macrophage polarization and double staining of macrophages with pre-collagen markers showed their active collagen synthesis as early as day 3. The control wounds treated by normal saline or Regranex^{TM} did not show similar changes.

CONCLUSIONS-We conclude that intracellular ATP delivery causes rapid tissue regeneration by initiating the wound healing cascade as early as 5hr. All the cellular events associated with this healing process may provide a new strategy for wound healing.

P.AW08

A Retrospective Review Of UBM-ECM Use In Treating Acute Plantar Wounds

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This study reviews our recent clinical experience using UBM-ECM (Urinary Bladder-Extracellular Matrix) to treat and achieve healing of clinically challenging acute plantar foot wounds ranging from a thru and thru heel pad wound to a total foot degloving wound from the level of the ankle.

Methods- We performed a retrospective review of the last 10 cases of acute plantar foot wounds treated with the use the UBM-ECM wound device. There were 8 males and 2 females with ages ranging from 15 - 66 years of age. There were varying degrees of tissue loss and depth of injury.

Results- Initial healing was achieved in all cases with 8 patients having either split or full thickness skin grafts used for final closure. NPWT was used while wounds drained 25 cc or more per day. Three heel pad patients developed secondary small wounds which were treated with additional UBM-ECM with 2 patients having achieved stable healing and the third patient with a recent opening responding well to treatment despite him standing at work over 60 hours a week. No patient required long-term narcotic use and all patients except the totally degloving foot patient have been able to resume all pre-injury activities. The total degloving wound patient had return of protective plantar sensation

We believe that the early use of UBM-ECM in the management of these potentially devastating plantar wound patients offers new hope to managing these complex wounds. Our present clinical treatment regimen and treatment outcomes will be presented. We strongly advocate early UBM-ECM use in managing these potentially devastating foot wounds.

P.AW09

Secondary Intention Healing After Surgical Excision Of Hidradenitis Suppurativa

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BACKGROUND - Hidradenitis suppurativa (HS) is a chronic, debilitating skin disease, characterized by recurrent inflammatory boils and abscesses, mainly located in the inverse body areas. Early wide surgical excision is important and effective in order to prevent complications, however, reconstruction is quite challenging, often requiring skin graft or skin flap. To evaluate the efficacy of secondary intention healing after wide excision for treating severe HS

METHODS - Over the last 10 years, 9 patients with severe HS (Hurley grade II and III) underwent surgical excision/ wide exteriorization with reconstruction using secondary intention healing and artificial collagen insertion. We evaluated and compared intraoperative and post-operative data, retrospectively.

RESULTS - Seven patients (77.77%) showed no recurrence after surgery. The mean treatment time to complete wound healing was 115±110days(range, 36-339days). The mean disease -free duration after treatment was 665±283 (range, 123-924 days).

CONCLUSIONS - Based on our experience, secondary intention healing after excision is an effective treatment option for patients with severe HS presenting multiple interconnected tracts and abscesses

P.AW10

Application Of Negative Pressure Wound Therapy After Distal Digit Amputation For Subungal Melanoma

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Background: The objective of this study is to evaluate the efficacy of the negative pressure wound therapy after distal digit amputation for invasive subungal melanoma

Method: Retrospective review of 11 patients (3 males, 8 females) from December 2015 to April 2017 was performed.

Results / Discussion: Mean age of the patients was 54.6 years (32~75 years) and all patients were diagnosed with SUM with deep invasion (Breslow thickness range : 1.5 ~ 5.0 mm / mean thickness : 2.85 mm). Index finger was involved in 4 patients, thumb in 3 patients, great toe in 2 patients, third finger in 1 patient, and second toe in 1 patient. The open amputation site was covered with fillet flap utilizing the volar skin and NPWT was applied for an average of 7.63 days (range : 3~14 days). The wound dressing period was 21 days (range : 12 ~ 37 days) and the flap survived successfully in all but 1 case (survival rate : 90.9%). In one case, the NPWT was stopped after 2 days due to pain of the perioperative margin and the flap did not heal well.

Conclusion: Distal digit stump is a difficult area to heal and ulceration is not uncommon complication. NPWT can be used effectively for flap survival and thus to shorten the duration of treatment after distal digit amputation. Since the stump is covered with thick and robust flap pad, functional and aesthetic outcomes are excellent.

AGING & SENESCENCE

P.AS01

Knowledge And Practice Of Diabetic Foot Care In Nursing Home Care Workers

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BACKGROUND: This study aimed to provide basic data for the design of education program about diabetic foot care for nursing home care workers. The purpose of this study was to identify knowledge and practice of care workers toward the diabetic foot care and to evaluate the factors affecting practice of diabetic foot care. METHODS: The research design was a cross-sectional study using a structured questionnaire. The participants were 90 care workers who were working in three nursing homes in J city from September to November 2016. RESULTS: Mean score of knowledge for the participants was 8.77 (range 6-10) and mean score of practice was 28.17 (range 20-30). Positive correlation was observed between knowledge and practice (r=.33, p=.002). Practice was significantly predicted by knowledge (p=.017) and received education on diabetic foot care (p=<.001) which explained 20.4% of the variance in practice. CONCLUSIONS: Knowledge and received education on diabetic foot care were found to be very important factors associated with practice of diabetic foot care in nursing home care workers.

ANGIOGENESIS

P.ANG01

Optimizing Healing Outcome By Zonal Conditioning Of Cutaneous Wounds Prior To Scar Formation Demonstrated In A Double-Blind Randomized Study

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BACKGROUND The concept of immediate versus delayed application of active topical formulations in order to optimize healing prior to scar formation is new. We previously evaluated the effects of an active versus placebo, post-scar formation. METHODS The aim here was to assess the application of an active versus placebo at multiple time-points pre-scar formation around the injury zone in 62 subjects (Group A:immediate application from Day (D) 0 around wound site, Group B:delayed application at D14 directly to scar) using quantitative non-invasive devices and immunohistochemical (IHC) analysis in a double-blind randomized-controlled trial. RESULTS Group A had greater hydration from D0 to W1 (148Au to 154.5Au) compared to placebo (174.4Au to 128.0Au) ($p=0.002$). TEWL reduced from D0 to W2 (6.3Au to 4.3Au) versus placebo (8.5Au to 16.1Au) ($p=0.02$). Group B showed increased hydration with active (21.0Au) compared to placebo (0.1Au) at W1 ($p=0.028$). IHC analysis established that hyaluronic acid (HA) proteins were located predominantly in the epidermis with weaker expression in the dermis. HA marker area was higher at all time-points in both groups. This increase was greatest at W1 with active (38%) versus placebo (21%) ($p=0.03$). Blood-flow measured by dynamic-OCT increased in Group A compared to placebo from D0 (0.009Au, 00.068Au respectively) to W1 (0.106Au, 0.086Au respectively) ($p<0.001$) and W2 (0.09Au, 0.081Au respectively) ($p<0.001$). Blood-flow was lower with active compared to placebo at all time-points ($p=0.037$). This was supported by VEGFA, which confirmed lower marker area with active (25.7%) versus placebo (46%) at W1 ($p=0.02$). CD31 analysis displayed lower vessel density with active (60vessels/mm2) at W1 than placebo (89vessels/mm2) ($p=0.03$). CONCLUSIONS These findings provide evidence, for the first time, of zonal conditioning for the immediate application of an active around the zone of injury in order to optimize healing outcome prior to scar formation and maturation.

P.ANG02

Phenotypic Change In Angiogenic Fibrocytes In Planter Decubitus Ulcers In Rats

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Background: The angiogenic property of fibrocytes and phenotypic change of fibrocytes remains fully unknown in wound healing. We previously demonstrated the specific induction of angiogenic fibrocytes by basic fibroblast growth factor (bFGF) in rat skin wounds. We here examined change in marker expression of angiogenic fibrocytes during the course of angiogenesis in rat planter decubitus ulcers. Methods and Results: Double immunofluorescence staining in the normal region showed that capillary-like structures were almost composed of CD34+/procollagen I+ fibrocytes. However, in the ulcers, capillary-like structures composed of CD34 -positive/procollagen I-negative cells were markedly formed. The ulcers also showed enhanced expression of TGFβ1 and VEGF-A mRNA and reduced expression of bFGF mRNA, without any change in levels of PDGF mRNA expression, as detected by Real-time PCR. Conclusion: We previously showed that bFGF is required for vascular formation composed of the CD34+/procollagen I+ fibrocytes in wounds. Therefore, enhanced VEGF-A expression in the ulcers markedly inhibited the vascular formation composed of this type of fibrocytes through reduction of bFGF expression in the ulcers.

BIOENGINEERING/BIOMATERIALS

P.BIO01

Protease Modulation By An Ovine-based Collagen Extracellular Matrix Dressing In An In Vitro Model Representative Of In Situ Use

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Dysregulation of a broad spectrum of proteases due to persistent inflammation is widely accepted as a feature of chronic dermal wounds. It has been proposed that excessive proteolytic activities in wound fluid may play a role in preventing wound closure, suggesting that wound dressings with antiproteolytic properties may be beneficial. An ovine-derived decellularized collagen extracellular matrix (cECM) comprising both collagen and non-collagen ECM components including elastin, fibronectin, laminin and glycosaminoglycans was utilized within this study. Here we describe an in vitro model designed to mimic the in situ use of this ovine dressing, whereby samples of ovine dressing were placed in microwell plates and real-time enzyme kinetics studies were performed for up to 6 days on 5 proteases commonly found in wound fluid: matrix metalloprotease-1 (MMP-1), MMP-2, MMP-8, MMP-9, and human neutrophil elastase (hNE); these were incubated in the presence or absence of ovine dressing, with a fluorogenic substrate (520 MMP FRET Substrate XIV; Anaspec). The activity of all 5 proteases tested was modulated by incubation with the ovine dressing, with inhibition levels reaching 80-90% after as little as 6 hours of incubation and maintained for at least 6 days for most enzymes. The inhibitory effect was perhaps more gradual for MMP-8 and MMP-9; and the incubation times beyond 1 day could not be explored for MMP-2 due to a relative instability of this enzyme. These data support the use of this ovine dressing to address the proteolytic conditions of chronic dermal wounds as well as set a new bar for demonstrating modulation of protease activity by collagen wound dressings.

Percent Inhibition

	MMP-1	**MMP-2**	**MMP-8**	**MMP-9**	**hNE**
0	69.4	84.9	79.5	-11.7	67.4
(1h)	74.6	88.0	-20.8	31.9	75.2
6h	92.3	95.3	61.2	78.5	97.5
1d	91.8	84.5	79.2	88.0	97.3
2d	91.6	53.5	82.0	85.2	98.7
4d	94.6	50.2	85.2	81.3	98.1
6d	92.3	21.4	93.9	78.5	98.5

P.BIO02

Structural Properties Of Viable Lyophilized Placental Tissues

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Recently, a novel lyopreservation method for ambient storage of living tissue has been developed. In this study, the effect of the novel lyophilization on the components of the placental tissue was evaluated. Structural matrix, growth factors, and cell viability of amniotic (AM) and chorionic (CM) membranes, and umbilical tissue (UT) were evaluated using 4 placentas. AM, CM, and UT were divided into two parts to enable comparison between fresh and lyophilized tissue. Fresh AM, CM, and UT were analyzed immediately. Lyophilized samples were analyzed immediately post-lyophilization, and after 6 months of ambient storage. Histological analysis (Hematoxylin and eosin staining, Masson's trichome staining) was used to assess tissue structure. Multiplex arrays and ELISAs were used for evaluation of growth factors, and viability of cells in the tissues was analyzed using a LIVE/DEAD viability/cytotoxicity kit. Histologically, the matrix of fresh tissue was retained in rehydrated lyopreserved AM, CM, and UT. There were no significant differences in the concentration of growth factors (basic Fibroblast Growth Factor, Platelet-Derived Growth Factor, Angiogenin-1, Stromal cell Derived Factor-1, Iinterleukin-1Receptor Antagonist) and cell viability between fresh and lyopreserved tissues. The matrix, growth factors and viable cells of fresh tissue were retained in lyophilized samples stored for 6 months. Results show that the new lyopreservation method is applicable for AM, CM and UT, reproducible and provides extended tissue shelf life. This preservation method for ambient storage of living tissues has tremendous practical significance for both scientific and clinical applications.

P.BIO03

Preparation And Activity Of Nanocellulosic Materials As Protease Sensors And Sequestrants

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Background: Chronic wounds are a major clinical problem with an estimated 40 million people suffering from them worldwide, and one of the most costly healthcare problems today. 'Intelligent' dressings may be defined as materials that respond to specific changes in the wound environment to bring about a useful result. Considerable promise in the application of nanocellulosic hydrogels, aerogels, and nanocomposites as dressings has been demonstrated due to favorable properties that promote optimal moisture conditions. Here we present a series of modified nanocellulosic and cellulosic materials designed to, remove harmful proteases from a chronic wound while detecting protease levels. Methods: The biosensors were prepared by a method previously report (Edwards's et al. J. Biomat. Appl., 2017). To start the sensor/protease reaction 50 μL of human neutrophil elastase at various concentrations ranging from 2 - 0.015 U/mL were utilized. Material protease sequestration evaluation was performed in a like manner using a 96-well format by incubating 2 milligrams of material in a protease solution for 1 hour. Results: A correlation between zeta potential values and the degree of protease sequestration imply that the greater the negative surface charge of the nanomaterials, the greater the sequestration of positively charged neutrophil proteases. The biosensors gave detection sensitivities of 0.015-0.13 units/ml, which are at detectable human neutrophil elastase levels present in chronic wound fluid. Conclusion: The sensor portion is a fluorescent peptide-cellulose conjugate interchangeable on the surface of different semi-occlusive dressing motifs and sensitive to protease levels found in chronic wounds. The protease modulation portion is based on the degree of surface zeta potential required on the material surface to remove excess wound protease levels. The physical and interactive biochemical properties of the nano-based biosensors are suitable for interfacing with protease sequestrant prototype wound dressings. A discussion of the relevance of protease sensors and cellulose nanomaterials to current chronic wound dressing design and technology is included.

P.BIO04

Assessment Of Matricellular Protein Biomimetic Scaffolds In A Porcine Model Of Cutaneous Wound Healing

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BACKGROUND - Impaired skin healing is a significant and growing clinical concern, particularly in relation to diabetes, venous insufficiency and immobility. In previous research, we developed electrospun collagen based scaffolds for the delivery of periostin (POSTN) and connective tissue growth factor 2 (CCN2), matricellular proteins involved in the proliferative phase of healing. Addition of the scaffolds rescued delayed healing in db/db diabetic mice. The aim of this study was to investigate the effect of POSTN/CCN2 electrospun scaffolds on wound healing in a porcine model. METHODS - 2cm^2 full thickness wounds were created bilaterally in the back of the pigs and scaffolds implanted. Healing was assessed through closure kinetics, hydroxyproline content, and blood vessel density at 28 days post-wounding (N=6). RESULTS - Wound closure was reduced in the presence of the three scaffold treatments (bovine serum albumin (BSA), POSTN/CCN2, and galectin-3 (GAL3)) when compared to empty control wounds. This effect was attenuated with POSTN/CCN2 and GAL3 scaffolds. Masson's Trichrome staining showed that collagen content was reduced by BSA, POSTN/CCN2, and GAL3 scaffold treatments but was near unwounded tissue levels in the empty wound condition. Blood vessel density increased in healing wounds with similar values between all scaffolds and empty treatments. CONCLUSIONS - Delayed contraction indicates scaffold influence over fibroblast to myofibroblast transition, potentially reducing excessive collagen deposition. These results indicate no negative effect of the electrospun scaffolds on blood vessel density. Future research is necessary with a larger sample size to validate the current findings.

P.BIO05

Assessment Of Human Amniotic Tissue Cell Viability

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Human amnion (hAM) has a long history of use for wound management. hAM is anti-inflammatory, anti-microbial and anti-fibrotic. It provides pain relief, keeps wound moist and supports angiogenesis, granulation tissue formation, and re-epithelialization. These beneficial properties are attributed to hAM components: structural matrix, growth factors, and viable cells. Advancement in preservation technologies led to hAM commercialization.Nevertheless, majority of the preservation methods destroy viable cells resulting in acellular or devitalized hAM. Cryopreservation is a method allowing retention of viable cells in hAM. However, data show that percent of viable cells in hAM post-thaw are not always consistent. Therefore, cell viability is an important test to ensure consistency and quality of cryopreserved hAM. Using fresh hAM samples of different sizes from multiple donors we evaluated several cell viability methods. We found that the LIVE/DEAD fluorescent staining method is the most appropriate for assessment of cell viability in tissues. However, high variability in number and distribution of viable cells across hAM grafts can lead to unreliable results when microscopic fields, a traditional approach, is used for estimation of cell viability percentage. We demonstrate that for accurate assessment of hAM cell viability scanning of large macroscopic tissue areas should be performed instead of microscopic field assessment. Then, we utilized the developed approach for assessment of cell viability in cryo- and lyopreserved hAM. We showed that there were no significant differences in cell viability between fresh, cryo- and lyopreserved hAMs. The developed approach gives accurate cell viability results and recommended for assessment of cell viability of the amniotic and other tissues for confirmation that applied tissue processing protocols do not compromise cell viability.

P.BIO06

Towards Next Generation Maggot Debridement Therapy: Transgenic Lucilia sericata Larvae That Produce And Secrete A Human Growth Factor

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Background: Diabetes and its concurrent complications impact a significant proportion the US population and create a large financial burden on the American health care system. Maggot debridement therapy (MDT) is the FDA-approved application of sterile, laboratory-reared Lucilia sericata (green bottle fly) larvae to non-healing wounds, such as diabetic foot ulcers. Larvae secrete a mixture of excretions and secretions (ES) as part of extracorporeal digestion of dead wound tissue that inhibit bacterial growth and promote wound healing. Human growth factors stimulate cell proliferation and survival in the wound environment, promote wound healing, and have been investigated as a possible topical treatment for non-healing wounds. We have previously illustrated the characterization of an L. sericata strain that expresses and secretes the human growth factor PDGF-BB, under the control of an inducible system. Methods: To continue optimizing our expression system, we are currently characterizing gene expression in an exhaustive panel of larval digestive system tissues and isolating promoter candidates for driving tissue-specific expression. Results: Having identified a number of promising candidates, we are also currently broadening the applications of our expression system by engineering new effector candidates with utility in wound healing. Conclusions: Honing expression to specific tissues will result in a lowered fitness cost to the organism and enhance efficiency of exogenous factor secretion. Our system could potentially be used to deliver a variety of growth factors and anti-microbial peptides to the wound environment with the aim of enhancing wound healing. Further, rearing maggots is relatively inexpensive, and could provide a cost effective treatment to patients in lower income regions.

BURN WOUNDS

P.BW01

Role Of Pressure Magnitude In Compression Garment Therapy

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BACKRGROUND: Pressure garments are commonly employed to reduce scarring following burn injury, with varying efficacy rates reported. The optimum magnitude of pressure necessary to achieve the greatest benefit is unknown, though it has been suggested that pressure should exceed capillary pressure, 25-30 mm Hg. Unfortunately, higher pressures are associated with increased risk of side effects such as deformation of skeletal features or constricted breathing. METHODS: To better understand the role of pressure magnitude on therapy efficacy, pressure garment therapy and scar development was studied in a porcine model. Full-thickness burns (1x1 in) were created on female, red Duroc pigs, excised, and autografted with split-thickness autografts. Adjustable pressure garments were applied 1 wk after grafting and maintained at either 10, 20, or 30 mm Hg for 11 weeks (n=16 scars/group). RESULTS: All pressure-treated groups were significantly less contracted than controls. Scars in the 30 mm Hg group were 56% larger than controls and 16% larger than the 20 mm Hg group at 11 weeks post-grafting ($p<0.05$). Pressure therapy, at all magnitudes, significantly improved scar elasticity and pliability, with the greatest improvements observed with 30 mm Hg. 30 mm Hg was the most effective at reducing scar contraction and improving biomechanical properties compared to 20 or 10 mm Hg. CONCLUSIONS: While pressure at a magnitude of 30 mm Hg resulted in the most significant benefit, the pressure is highly uncomfortable and would likely reduce the duration for which the patient can wear the garment. For greatest clinical efficacy, a balance between pressure magnitude and patient compliance must be achieved.

P.BW02

Scar Outcomes Following Pressure Garment Therapy Cessation

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BACKGROUND: The suggested duration of pressure garment therapy is commonly 1-2 years; however, maintaining patient compliance throughout this period is a major challenge. The goal of this study was to examine changes in scar properties after early cessation of pressure garment therapy. METHODS: Full thickness burns (1 x 1 in) were created on female Red Duroc pigs. Burns were excised and grafted with split-thickness autograft. Pressure garments were applied within 1 week and maintained at 20 $\pm$ 2 mm Hg. Treatment groups included: continuous pressure group, which received pressure for 29 weeks, pressure release group, which received pressure for 17 weeks, then pressure was removed for an additional 12 weeks; and a control group that did not receive pressure (n=8 scars/group). RESULTS: Pressure garment therapy significantly reduced contraction and scar height versus controls at 17 weeks post-grafting. When garments were removed, scars in the pressure release group rapidly contracted, with scar area at the conclusion of the study 22% greater than controls in the pressure release group and 86% greater versus controls in the continuous pressure group ($p < 0.001$). Scar height also increased 2-fold after pressure release ($p < 0.0$) and collagen fiber reorientation was observed after therapy was ceased. Pressure garments reduced scar height and contraction after 4 months of use; however, when therapy was stopped, scars rapidly contracted and became thicker. CONCLUSIONS: Pressure garment therapy must be continued until full scar maturation. Investigations into the change in scar behavior (gene expression, extracellular matrix production, cytokine production) upon garment removal are underway to identify key regulators of scar suppression via pressure therapy.

P.BW03

Kinetics Of Collagen Deposition In Burn Wounds Of Red Duroc Vs. Yorkshire Pig

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Background- Hypertrophic scarring (HTS) is characterized by firm, raised lesions, and excessive accumulation of collagen, and is one of the adverse outcomes of dermal burn injuries. HTS results in contractures that cause negative functional and aesthetic consequences while hindering full recovery and quality of patient's life. The scarring is thought to be a result of excessive collagen accumulation and rearrangement and is one of the frequently used as the clinically relevant endpoints for the measurement of HTS in different animal burn models. However, the kinetics of collagen accumulation during HTS formation in burn wounds over time and different anatomical location are not well determined. Method- Using a red Duroc porcine HTS model, we studied the kinetics of collagen accumulation comparing deep partial-thickness (DPT) to shallow burns spatially and temporally. Additionally, we also compared collagen kinetics of these burn wounds in the Yorkshire pig, which is less prone for scarring. Collagen levels were determined by total collagen assay and expressed as µg collagen / gram of freeze dried wound tissue. Results- Compared to shallow burns we detected a decrease in total collagen in DPT burn wounds for the middle region of the back of the Yorkshire pig during the first 30 days after burn. Thereafter, by post-burn day 60, DPT wound collagen levels returned to levels similar to those of shallow wounds and normal skin. On-going studies will determine the collagen kinetics of burn wounds in red Duroc pigs. Conclusion- Based on our current work, collagen levels per gram of wound tissue may not be an optimal clinical endpoint for HTS in the porcine animal model. Disclaimer: The opinions or assertions contained herein are the private views of the author and are not to be construed as official or as reflecting the views of the Department of the Army or the Department of Defense.

P.BW04

Improved Healing Of Deep Partial Thickness Burn Wounds With Omega-3 Rich Fish Skin Dermis Compared To Fetal Bovine Dermis

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Background: The paucity of donor sites in patients with burns involving large total body surface areas highlights the need for better cellular and tissue-based products (CTPs) that can achieve complete coverage without the need to graft. The purpose of this preclinical trial was to evaluate two xenogenic CTPs on deep partial thickness (DPT) burn wounds. Methods: DPT 5x5 cm burn wounds were created on the dorsum of anesthetized Yorkshire pigs using appropriate pain control methods. After 24 hours, wounds were excised to a viable wound bed and treated with fish skin graft (FSG, N=12) or fetal bovine dermis (FBD, N=12). FSG was reapplied after 7 days and all wounds were allowed to heal by secondary intentions for 60 days. Quantitative measurements include epithelialization, contraction rates, transepidermal water loss, hydration, and blood flow. Results: The wounds treated with FSG resulted in faster epithelialization (50.2% vs. 23.5% at day 14 and 81.7% vs. 62.3% at day 21, $p<0.001$). The FBD took longer to integrate into the wound bed than the FSG which was evident in higher hydration values at day 21 (2500.4 vs. 309.7 µS, $p<0.0001$) and lower blood flow measured at day 14 via laser speckle (3.3 vs. 5.1 fold change increase over normal skin, $p<0.0001$). Conclusions: Our results indicate that FSG integrated faster and allowed quicker wound closure without grafting while not increasing contraction of burn wounds. These results suggest that FSG could be an improved CTP that can promote healing of burn wounds while eliminating grafting requirements.

P.BW05

Successful Treatment Of A Severe High-voltage Electrical Burn With Thoracoabdominal Injury

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BACKGROUND Thoracoabdominal injuries often occur in association with multiorgan injuries. Here we present a case of severe high-voltage electrical burn with thoracoabdominal injury and share some experience of clinical treatment. METHODS A 60-year-old man suffered a severe work-related electrical injury caused by high-voltage current (10,000V). On admission, the patient presented progressive dyspnea with left chest wall defect. The full-thickness burn was 15% of his body surface area. Thoracoscopy exploration performed immediately. Diaphragm injury didn't found. The burn wounds were debridement, covered with artificial skin. Continuous negative pressure (125mmHg) was applied. On day 5, there was large amount of drainage in the chest tube. CT indication of colonic perforation. We detected perforation on splenic region of the colon and rupture of diaphragm. The gastrointestinal surgeons performed colostomy. The thoracic surgeons repaired the diaphragm injury with autologous dermis. On day 7, Splenectomy and partial pancreatectomy were performed because of splenic vein rupture and pancreatic tail necrosis. On day 16, high concentration amylase in intraperitoneal drainage fluid demonstrated pancreatic fistula. The burn wounds were cured by skin grafts and local flaps. Individualized negative pressure wound therapy helped the abdominal wound healing. RESULTS The patient discharged after 119 days. CT showed that the left lung was reexpansion and the fibrous adhesions formed diaphragm-like structure. Three months later, the colostomy closure was performed. After 6months of follow-up, the patient had no symptoms of discomfort. CONCLUSIONS Delayed necrosis of electrical burns is mostly unexpected. Physical examination combined with imaging methods and laboratory tests permit early diagnosis of severe injuries. Multidisciplinary treatment will reduce morbidity and mortality. Negative pressure wound therapy plays an important role in surgical treatment, especially in severe patients.

CHRONIC WOUNDS

P.CW01

Ectopic Expression Of Myocardin-related Transcription Factors Restores Myofibroblast Function In Chronic Wound Fibroblasts

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Background: Non-healing chronic wounds can lead to morbidity and mortality in patients. Chronic human wound fibroblasts (CHWF) are known to have deficits in cell proliferation, migration, and contraction. Our previous studies have demonstrated that the myocardin-related transcription factors A and B (MRTF-A/B) are involved in myofibroblast formation and function during normal cutaneous wound healing. We hypothesize that MRTF-A/B activity is reduced in CHWFs and that ectopic expression of MRTF-A/B will promote myofibroblast function in CHWFs. Methods: CHFWs were obtained from Coriell Institute for Medical Research and normal human dermal fibroblasts (NHDF) were obtained from Lonza. Whole cell lysates from CHWFs infected with lentiviral vectors to overexpress MRTF-A, MRTF-B, and LacZ and from NHDF infected with lentiviral vectors expressing either shRNA targeting MRTF-A/B or non-targeting shRNA were subjected to Western-immunoblotting for MRTF-A, MRTF-B, smooth muscle alpha actin (SMαA), SM22α, collagen type III, and GAPDH. Whole cell lysates of CHWFs and NHDFs were subjected to Western-immunoblotting for SMαA, MRTF-A, MRTF-B, SM22α, and GAPDH. Nuclear and cytoplasmic fractions from jasplakinolide (induces actin polymerization) treated CHWFs were subjected to Western-immunoblotting for MRTF-A, MRTF-B, Tubulin, and Lamin A/C. Lastly, contractile properties of MRTF-A/B depleted NHDF cells were analyzed in a stressed-relaxed collagen lattice contraction assay. Results: Ectopic expression of either MRTF-A or MRTF-B in CHWFs induced the expression of pro-contractile genes SMαA, SM22α. CHWFs expressed less SMαA and SM22α compared to NHDF. Jasplakinolide-induced actin polymerization in CHWFs promoted increased nuclear localization of both MRTF-A and MRTF-B. Depletion of MRTF-A/B in NHDFs reduced the expression of SMαA, SM22α, collagen type III and reduced stressed-relaxed collagen lattice contraction. Conclusions: These results suggest that decreases in MRTF-A/B activity results in a CHWF phenotype and that ectopic expression of MRTF-A/B in CHWFs can induce myofibroblast formation and function suggesting that MRTFs may be a therapeutic target to promote better wound healing in chronic wounds.

P.CW02

Modified Keystone Flap With Fortune Cookie Design: New "Workhorse" Flap In Gluteal Region

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Background The reconstruction of extensive soft tissue defects in gluteal region is a great challenge. Here, we describe our surgical experience of using modified keystone flap. Methods We retrospectively reviewed the data of 13 consecutive patients who underwent soft tissue reconstruction with modified keystone flaps between March and December 2017. (figure.1) Results Soft tissue reconstruction was performed without any major complications in the follow-up period. The reconstructed defect had a mean width of 6.3 cm and mean length of 7.3 cm, whereas the flap had a mean width of 8.3 cm and mean length of 12.1 cm. We have not experienced any partial flap congestion or necrosis. All patients were satisfied with the aesthetic outcomes.(figure.2-3) Conclusions Considering its simplicity, versatility, and reliability, our technique of using modified keystone flaps could be a reasonable surgical option for soft tissue reconstruction in gluteal region.

P.CW03

Generation Of An Extremely Long Term Ischemic Wound Model

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BACKGROUND - One major obstacle in chronic wound research is the lack of appropriate animal model with long ischemic time to recapitulate the primary pathophysiology of non-healing wounds. The purpose of this study was to create a significantly extended ischemic time in rabbits.METHODS - Twenty young adult New Zealand White rabbits were used. Employing the minimally invasive technique, three skin incisions were made on one ear base to ligate the central and cranial arteries, and a circumferential tunnel was created to disrupt the subcutaneous tissues. A medical grade silicone rod 3-5 mm in diameter was threaded through the tunnel but punch holes were made for the central and caudal veins. Tissue perfusion was measured daily for the first two weeks and then weekly using laser speckle Doppler imaging, TiVi8000 Tissue Viability Imaging system, a thermal imaging camera, skin temperature, and subcutaneous oxygen tension/saturation. RESULTS - Postoperative recovery was similar to the regular ischemic operation. In some rabbits, the ischemic ear showed edema for a few days. The implanted rod can be seen clearly on the ear base. Ear tissue perfusion was significantly reduced for more than 3 months in most rabbits with the longest one lasted for 10 months. Histology shows that, the implanted silicone rod occupies almost all the subcutaneous tunnel space, making it almost impossible for major collateral formation. No repeated surgeries were needed to maintain long-term ischemia. CONCLUSIONS - This new rabbit ear model provides a reliable extended ischemic period, closely resembling many types of human chronic wounds. It will be a valuable tool for chronic wound research.

P.CW04

Silver Bandage Toxicity To Human Skin Cells And To Leg Ulcers

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We investigated toxicity of bandages for wound healing with silver (three brands) or octenidine and hyaluronic acid (OCT-HA). The bandages were investigated in viability assays of keratinocytes and fibroblasts (NHEK, NHDF, respectively) and cell death flow-cytometry assay (all n=4). Ex vivo porcine skin was cultivated with the bandages on dermal side (n=4). Silver penetration was visualized with autometallography in porcine skin sections. γH2A.X was detected with immunofluorescence in the porcine skin and with WB in the skin homogenates. Gene expression was measured with qPCR (n=4). Patients (n=7) who gave a written consent were treated concomitantly on different parts of leg ulcers with silver bandage or OCT-HA. Biopsies were collected at the beginning, after 2 and 6 weeks and evaluated with histology - autometallography, trichrome stain and naphthol chloroacetate (granulocytes). Cell viability of both NHEK and NHDF treated with eluates from silver bandages was significantly decrased ($p<0.05$, t-test). NHDF died by necrosis after treatment with the eluates for 24 hours unlike the cells treated with OCT-HA eluate. Porcine skin incubated with silver bandages (27 or 51 hours) exhibited significantly higher DNA damage (elevated γH2A.X, $p<0.05$, t-test) and expression of stress genes HSPH1 and DNAJA1 ($p<0.05$, t-test). Silver penetrated deep into the ex vivo and in vivo tissues. Wounds treated with silver bandage had more granulocytes and inferior collagen maturation (both $p<0.05$) compared to OCT-HA after 2 weeks. High silver penetration, toxicity and no biodegradability impose unnecessary burden on the wounds. Therefore, use of silver bandages may slow down wound healing and one should consider safer alternatives.

P.CW05

Clinical Assessment Of A New Biofilm Disrupting Agent For The Management Of Chronic Wounds Compared To Standard Of Care - A Novel Approach

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Chronic ulcers harbor a plethora of microorganisms that are resistant not only to conventional wound care but also to physical debridement, topical therapies and dressings in addition to multidisciplinary treatment strategies. The presence of biofilms in chronic wounds, present in over 80% of patients with infection, is a significant obstacle to wound closure. Current topical applications are not effective in treating bacterial biofilms in wounds.

To determine if disrupting chronic wound biofilm would be therapeutically efficacious, we studied the use of a novel topical agent for wound management, specifically targeting biofilms. 36 patients with chronic recalcitrant wounds were randomized to a 12-week treatment with a broad spectrum antimicrobial ointment or a biofilm disrupting wound gel. Wound healing rate was assessed by measuring wound size reduction and closure rates.

Wound size decreased significantly with a 71% reduction in wound area for wounds treated with the biofilm disrupting gel, compared to 24% for the control ($p < 0.001$). Wound closure was attained in more than half of the patients treated with the test product. 53% of these patients achieved closure by 12 weeks, as opposed to 17% for the control ($p < 0.01$). There were no adverse events related to the biofilm disrupting product while two adverse reactions occurred with the control.

The combination of the novel biofilm disrupting agent with wound debridement, significantly improves wound healing rates by disrupting the biofilm which protects multispecies bacteria within a chronic wound. Given the significant wound size reduction and closure rates observed in these long-term non-healing wounds, and a lack of related serious adverse events, the biofilm disrupting wound gel, in our setting and experience, is a safe and effective treatment for recalcitrant chronic wounds.

P.CW06

Treatment of Deep Cavities Using a Perforator-Based Island Flap with Partial De-Epithelization

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Purpose: The perforator-based island flap is a popular option for defect coverage. In cases with deep cavities, however, the classical island flap may not be a suitable option. By de-epithelization of the peripheral portion of a perforator-based island flap, the distal part of the flap can be used to fill deep spaces, as the flap can be folded and inserted into the spaces. Methods: From June 2015 to April 2017, 21 cases of deep internal defects were reconstructed with perforator-based island flaps with peripheral de-epithelization. A fasciocutaneous flap was elevated and rotated with the pivot point on the perforator traced by hand-held Doppler. After measuring the size of internal space, de-epithelization was performed on the periphery of the flap. The de-epithelized portion of the flap was inserted and anchored into the internal defect (Figure 1 & 2). Demographic information about the patients, the size of the defects, the perforators that were used, and complications were recorded (Table 1). Results: During the follow-up period (mean, 14.2 months) of total 21 cases (16 pressure sores, 2 meningomyelocele defects, and 3 cutaneous fistulae), no major complications such as flap loss occurred. In 2 cases, a minor complication was observed. Temporary flap congestion was seen in 1 case, and was treated with a short period of leech therapy, and the other case was partial necrosis on the flap margin, which was cured with minimal debridement and conservative treatment. No major problems have occurred, especially on the de-epithelized part of the flap and in the occupied space. All deep cavities were completely reconstructed. Conclusion: A perforator-based island flap with partial de-epithelization can be a useful option for the surgical treatment of deep cavities. With performing safe procedure, the flap can be transferred safely without anxiety regarding the buried flap.

P.CW07

A Murine Model Of Aged Diabetic Wound Healing

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Background - Chronic diabetic wounds impact over 3 million Americans per year and cost more than $9 billion to treat on an annual basis. With the concomitance of an increasingly aging population the deleterious effects of diabetic wounds are becoming even more dramatic in the elderly, as aging represents an independent, synergistic risk factor for impaired wound healing. This scenario points to the need to develop reliable preclinical models of aged diabetic wounds with the goal of studying the unique patho-physiology of this condition and the effectiveness of novel therapeutic approaches. Methods - Here we adopted an established murine model of type-2 diabetes (db/db) and investigated the varying biology of wound healing in young (10-weeks old) and old (12-months old) animals (n = 6 /group). A dorsal wound was performed on each animal and covered with a transparent dressing. Digital pictures and tissue specimens of wounds were obtained on day 0,5, and 10. Histological samples were used to measure granulation tissue formation, inflammation, cell proliferation and angiogenesis. Anova with post-hoc Bonferroni correction was used for statistical analyses. Results - In the db/db model aged animals showed a moderate by significant slower wound closure rate compared to younger mice ($p=0.05$). Histology confirmed significantly lower granulation tissue formation, pathologic collagen remodeling, and decreased angiogenesis in the older animals compared to their younger controls. Conclusions - The aged db/db mouse might represent a preclinical model to study the synergistic effects of aging and type-2 diabetes in chronic wound healing. This model should be further characterized and implemented to help design effective clinical therapies.

P.CW08

Dehydrated Human Amnion Chorion&Allograft Improved Diabetic Cutaneous Wound Healing

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Background: Wound healing is a multiple cellular processes including proliferation, migration, and angiogenesis, which are severely interfered under diabetes mellitus. Amnion was clinically demonstrated to enhance the healing of chronic diabetic foot ulcers as it contains numerous cytokines, growth factors and hormones well known to be potential to facilitate wound healing processes. However, the pathways through which these biological components of the placental tissue promote diabetic cutaneous wound healing are highly required to be elucidated. Utilizing diabetic splinted excisional wounds murine model established previously in our laboratory, the effect of dehydrated human amnion/chorion allografts (dHACAs) on diabetic wound healing was investigated in this study. Methods: Splinted, full thickness excisional wounds were created on the dorsal skin of *db/db* mice, and immediately applied with dHACAs topically or covered with dressing alone. The wounds was tracked and harvested on post operation day (POD) 7 and 14 for further measurement healing parameters including re-epithelialization, granulation tissue deposition, proliferation, angiogenesis and immune-inflammatory responses through histological analysis, immunohistochemistry and PCR array. Result: Compared to the primary dressing alone, dHACA notably promoted the re-epithelialization rate, and deposition of granulation tissue in wounds as showed in histological analysis. Immunohistochemistry staining displayed that the expression proliferation (Ki67) and angiogenesis (CD31) in the diabetic wounds were also enhanced by dHACA administration. The ratio of remodeling/wound healing M2 to proinflammatory M1 macrophages was also increased in the dHACA treated wounds. PCR array data indicated that the genes related to angiogenesis, keratinocyte migration, proliferation, and inflammatory response were regulated by the administration of dHACA. Conclusions: These data demonstrated that amnion/chorion membranes promoted diabetic wound healing through enhancing cellular proliferation, migration and angiogenesis, as well as regulating immune-inflammatory response of the host.

P.CW09

Prospective Clinical Observational Study On Efficacy Of Dichloride Octenidyne In The Treatment Of Chronic Wounds

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Introduction Chronic wounds (CW) have constituted a very serious medical problem in recent years especially when critically colonized or infected, they need special preparation for future treatment, especially possibility of use of antiseptics which are sometimes controversial.

Study aim

An assessment of the efficacy, versatility and effect of dichloride octenidyne (DO) on the changes of clinical condition of CW.

An assessment of effect on bacteriological status of CW, changes during treatment with particular evaluation of alert-pathogens

An assessment of risk of local and general intolerance and side-effects.

Material and methods The prospective clinical study was conducted from May 2015 to May 2017 in 188 patients with different CW: pressure ulcers, venous leg ulcers, diabetic foot and ischemic ulcers. The wounds were clinically assessed for the amount of necrotic tissue, granulation, epithelisation, amount of exudate, pain level and antimicrobial status.

Results In the patients with CW the clinical assessment was carried out in all patients at the beginning after 4 weeks of the treatment. Significant changes were observed in all the studied parameters -reduction of necrotic tissue, exudate and reduction pain, increased granulation ($p<0.05$). The statistical analysis of the changes of bacterial counts at the beginning and after 4 weeks of the treatment with DO demonstrated changes and reduction of bacterial count ($p<0.05$).

Conclusions

DO is effective and useful, caused statistically significant changes in clinical condition of CW which can prepare wound for future treatment. There were observed high activity of octenidyne against bacteria, Gram-negative and Gram-positive isolated from CW. It caused eradication of multiresistant strains (89%) and bacteria qualified as alert-pathogens (100%).

No side effects and significant local intolerance reactions used was observed. The DO used proved to be very safe.

P.CW10

The Combined Effect Of Mesenchymal Stem Cells And Chicken Embryo Extract On The Flap Viability And Mast Cells In Random Skin Flaps

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The aim of present study was to investigate the effect of bone marrow mesenchymal stem cells (BMMSCs), and chicken embryo extract (CEE) on the viability, blood vessels number, and mast cells(MCs) number and degranulation of random skin flaps (RSFs). A 30×80 -mm RSF were made in dorsum of forty rats. They were divided into four groups as follow. Group 1 was served as control, group 2 received BMMSCs, group 3 received CEE+ BMMSCs, and group 4 received CEE. BMMSCs and CEE were injected to the flaps immediately after surgery. Seven days after surgery, survived part of flaps were measured, and blood vessels sections, mast cells number and degranulation were examined. We observed significant increase in survival area of flaps, and blood vessel sections of all treatment groups compared to control group. BMMSCs group showed significant decreases in survival part of flap compared to CEE and CEE+ BMMSCs groups. Moreover type two MCs (complete degranulation state of MCs) and total number of MCs in BMMSC group were significantly higher than other groups. The stimulatory effect of BMMSCs, CEE, and CEE+BMMSCs was presented by significant increase in survival part of flaps compared to the control group. And CEE group was more effective statistically compared to the BMMSCs, and BMMSCs+CEE groups. We hypothesized that MCs productions induced an inhibitory effect on survival of the RSFs.

P.CW11

Clinical Assessment The Efficacy Of A Biofilm Disruption Wash In The Treatment Of Chronic Wounds: A Pilot Study

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Chronic ulcers harbor a plethora of microorganisms that are resistant not only to conventional wound care but also to physical debridement, topical therapies and dressings but also to multidisciplinary treatment strategies. The presence of biofilms in chronic wounds, present in over 80% of patients with infection, is a significant obstacle to wound closure. Current topical applications are not effective in treating bacterial biofilms in wounds.

The use of a novel biofilm disruptive wash that is broad-spectrum with low to no toxicity to the host tissue is a very promising approach to solving the chronicity of diabetic wound ulcers. The objective of this 10-subject prospective pilot study is to assess the reduction in the bacterial load within the wound by DNA typing after the use of the biofilm disrupting wash post-debridement and the associated reduction in size or closure of chronic wounds over 12 weeks

In our study, we were able to quantify bacteria for all samples pre- and post-debridement. For all patients, there was a measurable decrease in the bacterial load within the wound after treatment with the lavage. The average wound size reduction for the ten patients over the first four weeks of treatment using the wash after debridement was 57%, with 6/10 patients achieve greater than a 50% reduction in the first four weeks of treatment. These patients will need to be followed to determine the wound healing rate for and the correlation of bacterial burden to wound healing. A larger sample size study will need to be performed to achieve statistical significance of the correlation between the use of the biofilm disrupting wash and its effect on wound healing.

P.CW12

Immunomodulatory And Wound Healing Effects Of Combined Chlorogenic Acid And Myricetin Formulation

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Natural compounds have a significant potential to treat wounds caused by trauma, diabetes, ischemic syndromes and other pathological diseases. This is due to their antioxidant, anti-inflammatory and anti-bacterial characteristics. Wound healing complexity necessitates the use of multi-target drugs with immunomodulatory, pro-angiogenesis and pro-healing properties. In this context, polyphenols have received increasing attention due to their low toxicity and potential to alleviate symptoms and inhibit the development of various skin disorders, skin aging, and skin damage, including wounds and burns. In this work, we explored the potential of a flavonoid, myricetin-3-*O*-rhamnoside, and a phenolic compound, chlorogenic acid, both isolated from *Parrotia persica* leaves, to suppress inflammation, promote wound healing and accelerate angiogenesis. This was accomplished using *in vitro* scratch and tube formation assays utilizing human epidermal keratinocytes (NHEKs), human dermal fibroblasts (NHDFs) and human umbilical vein endothelial cells (HUVECs). We also investigated the influence of these compounds on driving pro-inflammatory environment to anti-inflammatory environment to accelerate wound closure of fibroblast using conditioned media of macrophages. The assessment of dose response of the compounds demonstrated no cell toxicity between the dosages of 1 µg/mL to 100 µg/mL. Wound closure as early as 6 hours, demonstrated approximately 40% of the gap to be closed with 10 µg/mL of chlorogenic acid. On the contrary, myricetin was most effective in promotion of wound closure in assays using NHDFs (3-fold increase) as compared to negative control. Both compounds were able to induce tube formation at an approximately 50% higher rate as compared to control groups. Altogether, our results demonstrate the potential of myricetin and chlorogenic acid to be used in combination in treatment of cutaneous wounds.

P.CW13

Combining Treatment Methods For Diabetic Foot Ulcers - Tissue Base Products, Negative Pressure Wound Therapy And Offloading

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Background: In the treatment of diabetic foot ulcers (DFU), there are many treatment options, however, to address the pathophysiology, the most important consideration is offloading. Negative pressure wound therapy (NPWT) is one option to enhance healing. In practice when electrical power NPWT is applied, this usually precludes offloading with total contact casting (TCC). In this limited case series, we share our approach in combining cellular tissue based products, NPWT and TCC in the treatment of DFU. Methods: Three patients with refractory DFU's and multiple recurrences were treated using simultaneous application of a cellular tissue based product, mechanical power NPWT and TCC offloading. Results: These three patients demonstrated favorable clinical progress toward healing and were able tolerate this approach without significant difficulty or adverse event. Conclusion: Our preliminary impression is that combining these three treatment methods (cellular tissue based products, negative pressure wound therapy and TCC offloading) in the treatment of DFU is an extremely useful protocol. This multipronged approach simultaneously addresses several pathological features underlying the diabetic foot ulcer and could potentially lead to shorter healing times and less complications. These findings need to be replicated in other practices and at larger scale.

P.CW14

Combining Treatment Methods For Venous Leg Ulcers - Tissue Based Products, Negative Pressure Wound Therapy And Compression

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Background: In the treatment of VLU, there are many treatment options, however, to address the pathophysiology, the most important consideration is compression therapy (1). Negative pressure wound therapy (NPWT) is one of the options to enhance healing. In practice when electrical power NPWT is used, usually it precludes compression therapy. In this limited case series we are sharing our approach in combining multiple treatment methods for VLU patients and to enhance benefit of cellular tissue products. Methods: Three patients with refractory VLU and multiple recurrences were treated with this approach, using simultaneously a tissue based product, mechanical power negative pressure wound therapy and compression therapy. Results: These three patients had very satisfactory clinical progress towards healing and were able to tolerate this approach without significant difficulty or adverse events. Conclusions: Our preliminary impression is that combining multiple treatments methods simultaneously (tissue based product, NPWT and compression) is an extremely useful treatment protocol. Potentially this can lead to shorter healing time and less complications. These finding need to be replicated in other practices and at larger scale.

P.CW15

Early Detection Of Deep Tissue Injury In Critically Ill Surgical Patients

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Background: The purpose of this pilot study was to demonstrate the feasibility of using non-invasive optic measurements of blood flow as a screening tool for pressure injury (PI) in the surgical intensive care unit (SICU). Frequently, these injuries originate as deep tissue injury (DTI) several millimeters beneath the skin's surface. DTI is not clinically apparent until it spreads through subcutaneous tissue. By this time, the damage may be too extensive to avoid advanced ulceration.

Methods: Our long-term goal is to develop and validate a method for early detection of DTI by measuring blood flow in the dermis and subcutaneous tissue with a non-invasive optical method termed diffuse correlation spectroscopy (DCS).

Results: We recently completed a pilot study at a rehabilitation hospital suggesting that sacral blood flow measurements can detect sacral PI development prior to clinical appearance. For this study, we recruited 10 patients (60% male, 57.5 $\pm$ 13.13 years of age) from a SICU and gathered data on blood flow, clinical, nutrition and metabolic characteristics. We compared the data obtained from patients who developed PIs (N=1) with those who did not (N=9). Although only one subject developed a sacral PI during this study, the data obtained from this subject's sacral tissue suggested that an increase in local microcirculatory blood flow may have preceded ulceration.

Conclusions: These preliminary results and the logistic information obtained will be used in the design of a larger-scale clinical trial to determine the efficacy of DCS for early detection of PI in critical care populations.

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EPITHELIALIZATION

P.EP01

Novel Application Of Cultured Epithelial Autografts (cea) With Expanded Mesh Skin Grafting Over Artificial Dermis

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Backgrounds: Cultured epithelial autografts (CEA) is useful in epithelialization and contribute to successful life-saving in extended burns. CEA with highly expanded mesh skin grafts were used for extensive adult burns covering more than 30% of the total body surface area, which is regulated by the reimbursement system and sought to investigate the efficacy and benefit of this method. Methods: A prospective study on 8 patients assessed subjective and objective findings up to a 12-month follow-up after standardized debridement and temporal artificial dermis coverage. The wound healing for over 1:6 mesh plus CEA, gap 1:6 mesh plus CEA, and 1:3 mesh were compared at 3, 6, and 12 months using extensibility, viscoelasticity, color, and transepidermal water loss by a generalized estimating equation (GEE) or generalized linear mixed model (GLMM). Results: No significant differences were observed among the paired treatments at any time point. At 6 and 12 months, over 1:6 mesh plus CEA achieved significantly better expert evaluation scores by the Vancouver and Manchester Scar Scales. Extended skin grafting plus CEA minimizes donor resources and the quality of scars is equal or similar to that with conventional mesh slit-thickness skin grafting. Conclusion: This is a very unique clinical study focusing on the scar quality and cost-effectiveness of donor sites and a longitudinal analysis of scars may further clarify the molecular changes of scar formation and pathogenesis.

EXTRACELLULAR MATRIX

P.EXT01

A Retrospective Review Of UBM-ECM As A Primary Reconstructive Modality In Complex Wounds

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Porcine urinary bladder extracellular matrix (UBM-ECM) with its formulations as a micronized powder and sheets of varying thickness both lyophilized and vacuum-pressed has allowed for the use of these devices to move from an adjunct to topical healing to use as a primary reconstructive modality. Methods- We performed a retrospective review of our series of 353 patients with 429 wounds treated with UBM-ECM and identified 14 significant wound cases in medically complex patients where the wound device was used as a primary reconstructive modality in lieu of standard flap procedures. There were 9 males and 5 females with an age range of 24 - 80 years. There were 6 acute and 8 chronic wounds treated. Nine wounds had significant bacterial colonization at the time of treatment. There were 2 head and neck, 2 upper extremity, and 8 lower extremity cases. Results- Multiple UBM-ECM formulations were used in all case. Nine patients required multiple operative device applications and/or office applications. Skin grafts both split and full thickness skin grafts were employed at a secondary procedure in 3 cases. Secondary wound care involved negative pressure wound therapy with wounds draining over 30 cc/day. The time to healing varied and roughly correlated with the size of the wound. Compared to standard flap operative procedures, the use of UBM-ECM wound devices took longer to achieve healing but avoid donor sites other than skin grafts. These devices performed well in the face of multiple medical co-morbidities and bacterial colonization. No patient failed ECM treatment and had to be “salvaged” with a reconstructive flap procedure. We believe the use of the UBM-ECM wound devices offer a reasonable treatment alternative for medically complex wound patients who desire less complex treatments and should at least be mentioned to patients when reviewing possible treatment options.

P.EXT02

The Relationship Between Bone Biopsy And Bone Culture Results In Patients Withosteomyelitis From Pressure Ulcers

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Osteomyelitis is an infectious process of the bone caused by prolonged immobility, bacterial dissemination through blood, immunosuppression, trauma, or vascular insufficiency. The diagnosis of osteomyelitis is made by multiple modalities. Some of these include bone cultures, bone biopsy, imaging, and clinical determination[1]. In this study we plan to chronicle patients with suspected osteomyelitis secondary to prolonged immobility resulting in pressure ulceration. Our aim is to find correlations between bone cultures and biopsy findings of the bone. The relationship between the two entities can help guide extended antibiotic therapy.

FIBROSIS/SCARRING

P.FS01

Differential Regulation Of Exosome Production By Mechanical Tension Influences Fibrogenic Phenotypes

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Although it is known that fibroblasts promote scarring, differences in phenotypic variability and the signaling mechanisms between fibroblasts are yet to be defined. Here, we postulate that differential fibroblast responses to mechanical tension regulate exosome production.

We recruited patients who had given birth by caesarian section and were undergoing abdominoplasty procedures under IRB guidelines and pre-operatively evaluated them using the Vancouver scarring scale(VSS;n=8;29-55y.o. female;healthy and non-smoking). Skin and C-section scar samples were obtained from discarded tissues and evaluated by histological staining and immunohistochemistry(F4/80[macrophages]; CD3/CD69[T-cells]). Fibroblasts isolated from patients' normal skin and C-section scars were cultured on silicone membranes ±10% static strain(24hrs). qRT-PCR evaluated expression of fibrotic markers(CD26/alpha-SMA/TGF-B) and genes encoding exosome synthesis(RAB27a-b;SMPD3). Exosomes were analyzed for size and quantity(Zetasizer). Qualitative analysis was performed by three independent blinded investigators; in vitro data: mean ±SD;p by ANOVA.

Patient scars were classified as "low" or "high" based on VSS(<3 vs. >6). Histological staining revealed thicker collagen bundles/whorls(H&E), elevated collagen turnover(Herovici), and increased presence of F4/80 and CD3-positive cells($p<0.05$) in high-scar samples relative to low-scar and normal skin(n=3). Preliminary data from one patient's normal skin and C-section scar-derived fibroblasts, as well as high/low-scar derived fibroblasts, showed that tension increases expression of CD26(~2.8-fold), a-SMA (~3-fold), and TGF-B1(~2.3 fold) in all four groups; $p<0.05$;3 passages per cell-line. Notably, high-scar fibroblasts significantly increase expression of these markers relative to other groups($p<0.05$). In contrast, exosome synthesis genes(RAB27a-b, SMPD3) are down regulated (~2-fold; $p<0.05$) in all groups under tension. Exosome profiles are more variable: under static conditions, exosomes from normal skin and low-scar derived fibroblasts were similar, while those from high-scar derived fibroblasts were more abundant and larger in size($p<0.05$).

Our findings suggest that fibroblasts' differential response to tension includes changes in exosome production, which could influence fibrogenic phenotype. Elucidating the exosome profile/cargo could reveal fundamental biologic differences underlying heterogeneity in human scarring.

P.FS02

Quantitative Index For Skin Fibrosis: Combined Optical Coherence Tomography With Ultrasound Validated By Histology And Immunohistochemistry

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BACKGROUND The ability to characterize cutaneous fibrosis is of clinical and scientific relevance, as this is a critical step in assessing scarring severity, healing potential, and determining response to therapies across all wound and scars. A number of non-invasive objective technologies have been used to assess cutaneous fibrosis. Optical coherence tomography (OCT) and high-frequency ultrasound (HFUS) are real-time, non-invasive techniques that are used effectively for measuring physiological changes in tissue structure and function. We have previously compared OCT to histological assessment of acute wound healing and identified a novel measure of dermal fibrosis and remodeling known as the mean grey value. METHODS Our aim was to generate normative data using OCT and HFUS for all phases of healing with multiple sequential biopsies (day 0 and weeks 1,2,3,4,5,6,8) created in 62 healthy volunteers supported by histological and immune-histochemical analysis. RESULTS The attenuation coefficient values from OCT were significantly reduced from baseline (2.11/mm) to week 5 (1.43/mm) ($p<0.001$). Herovici staining demonstrated that mature collagen was greatest in normal skin (59%) compared to week 8 (24%) ($P=0.04$), whilst immature collagen was greater at all subsequent time points compared to baseline ($p<0.05$). This was further supported by additional collagen I and III analysis. HFUS measurement of skin thickness increased from week 1 (1.28mm) and slightly increased to week 6 (1.62mm), which was also confirmed by OCT measurements and H&E analysis ($p=0.004$). Elastin was significantly greater in normal skin (49.1%) compared to scar tissue with lowest values at week 4 (23.0%,$p=0.01$) and a gradual return to values similar to baseline by week 8 (41.4%). Fibronectin intensity was highest at week 4 (0.28Au, $p=0.02$) and reduced to week 8 (0.16Au). CONCLUSIONS OCT and HFUS provide a quantitative baseline for the assessment of dermal fibrosis, which may assist in the development of new theranostic strategies throughout therapy.

P.FS03

Topical Administration Of Liposome Encapsulated Statins Reduced Hypertrophic Scarring. A New Therapeutic Option

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Background: Hypertrophic scar is an important clinical problem with limited therapeutic options. Topical treatment by a repurposed medication already in widespread clinical use would be an important addition to the therapeutic armamentarium. Statins are a class of commonly prescribed cholesterol-lowering medications, and have also been demonstrated to inhibit fibrosis. We have been investing for many years statin treatment which acts by reducing CTGF expression as an option for hypertrophic scar. However, the low dermal penetrative ability of statins limited their topical treatments for hypertrophic scars. The objective of this study is to develop novel statin formulations with liposome which can enhance the dermal penetrative ability, and evaluate their efficacy on hypertrophic scar formation utilizing our validated rabbit ear hypertrophic scar model. Methods: Simvastatin (hydrophobic) and pravastatin (hydrophilic) were encapsulated in liposome using a novel flexible liposomal formulation. Hypertrophic scars formed after the cutaneous wounds created on rabbit ears healed completely on post operation day (POD) 14, and were daily treated with either liposomal simvastatin or pravastatin at 6.5% concentration topically until POD 25. The contralateral ear received liposome vehicle alone served as vehicle control. All the scars were tracked and harvested on POD28 for the evaluation of scar elevation index as well as gene expression related to fibrosis. Results: Topical treatment with simvastatin and pravastatin in liposome significantly reduced scar elevate index by 31.1±9.0% ($p<0.01$) and 35.0±8% ($p<0.01$) separately. The mRNA level of CTGF, TGFβ-1, collagen I and III in the scar tissue were also decreased by the liposomal pravastatin treatment. Conclusion: The novel statins formulations encapsulated in liposome were successfully delivered through topical application, and significantly reduced hypertrophic scarring in a rabbit ear model.

P.FS04

Altered Shear Forces Precipitate Fibrotic Remodeling in Discrete Subaortic Stenosis

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BACKGROUND-Discrete subaortic stenosis(DSS) is a congenital anomaly characterized by fibrosis in the left ventricular outflow tract(LVOT). Treatment is usually surgical intervention but is associated with significant recurrence. The pathogenesis of DSS is unknown because of a lack of appropriate models. It is postulated that altered shear stresses in the LVOT cause endothelial cell injury and a fibrotic cascade. Therefore, we have created a novel bioreactor to mimic intra-cardiac shear-forces and hypothesized that altered shear-stress is sensed by endocardial endothelial cells(EEC) resulting in inflammatory-mediated fibrotic tissue formation. METHODS-EEC and cardiac fibroblasts(CF) were isolated from porcine LVOT and subjected to normal and pathologic shear-forces based on DSS patient echocardiographic data. Using the parallel plate flow bioreactor, variable shear forces were applied to EEC monolayers and analyzed by immunocytochemistry(CD31;VE-Cadherin;ASMA) and PCR array. The conditioned media from the monolayers were then transferred to CF, and analyzed by PCR array. Human DSS tissues were obtained from the Texas Children's patient tissue-repository(n=7), and compared to dermal scar specimens by immunohistochemistry and histology. RESULTS-High shear-stress mimicking DSS caused the de-localization of mechanosensitive CD31 from the VE-Cadherin-rich cellular junctions in EEC. PCR array analysis revealed up-regulation of pro-inflammatory CCL3, CCL4 and CSF1 genes under pathologic shear(26.11, 14.38 and 5.07 fold regulation versus normal) and a significant upregulation of ASMA, a marker of endoMT($6.2\pm3.3, p<0.05$). Pathologic shear-conditioned EEC media resulted in upregulation of fibrosis and matrix remodeling genes(TGFb,col3a,Bmp7) in CF. Histology revealed phenotypic similarities between fibrosis in DSS tissues and dermal scars, including increased collagen remodeling(Herovici), disorganized collagen(Trichrome), and presence of activated fibroblasts(25% of resident cells, ASMA). CONCLUSIONS-Our data suggests altered shear forces affect mechanosensitive proteins in EEC and upregulate inflammatory and fibrosis related genes. Additionally, histologic characteristics of DSS mirror fibrosis in dermal scarring. Understanding the pathogenesis of DSS through mechanosensing may elucidate novel therapeutic targets for conditions characterized by fibrosis associated with altered mechanical forces.

P.FS05

Pirfenidone Inhibits Contractile Machinery In Profibrotic Human Dermal Myofibroblasts

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BACKGROUND - Pathologic dermal scarring such as hypertrophic scarring occurs when the normal wound healing processes of extracellular matrix deposition and remodeling become dysregulated. The profibrotic cytokine transforming growth factor beta 1 (TGF-β1) transforms fibroblasts in the wound bed into myofibroblasts with contractile F-actin stress fibers containing a smooth muscle actin isoform (α-SMA). Previous *in vitro* studies promoted pirfenidone as a potential dermal fibrosis prophylactic and established that concurrent treatment of normal human dermal fibroblasts with TGF- β1 and pirfenidone diminished the development of the contractile machinery elements of myofibroblasts. This *in vitro* study investigates the potential for pirfenidone treatment to mitigate an established myofibroblast phenotype.

METHODS - Normal human dermal fibroblasts were treated with pirfenidone after three-day stimulation by TGF- β1. Time course experiments were analyzed via immunocytochemistry and quantitative image analysis of F-actin stress fibers and α-SMA. Effects on cell contractility were assessed with fibroblast populated collagen lattice contraction assays.

RESULTS - Preliminary findings indicate that pirfenidone treatment of established myofibroblasts reduced F-actin and α-SMA in contractile stress fibers and reduced collagen gel contraction.

CONCLUSIONS - Pirfenidone is a potent antifibrotic capable of modulating the structure and function of the main effector cells of scarring, myofibroblasts.

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P.FS06

Characterization Of A New Variable Porosity Wound Dressing With Anti-scar Properties

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BACKGROUND: Excessive scar formation can be a devastating consequence of wound healing after injury from chemical or physical agent burns of the skin and other organs. There are few, if any, effective treatments to promote wound healing while effectively preventing scar formation. New treatments are needed. One option in topical wound healing is the use of temporary dressings that allow the natural healing process with minimal scar formation. METHODS: We evaluated PermeaDerm C (PDC), a biosynthetic variable porosity matrix that contains gelatin and Aloe Vera for chronic wounds, and PDC derivatives coated with the anti-scarring agent, salinomycin termed PermeaDerm A (PDA) for their ability to promote cell growth while preventing excessive scar-forming cell (myofibroblast) accumulation. Human and porcine fibroblasts and human stem cells grow favorably *in vitro* in the presence of PDC matrices. RESULTS: PDA attenuated TGFβ-induced scar forming myofibroblast formation of human and porcine cells. Additionally, both PermeaDerm matrices (PDC and PDA) significantly reduced the production of the inflammatory cytokines: IL-6, IL-8, MCP-1 and GRO. Specifically, PDC and PDA reduced IL-6 production by 50%, IL-8 by 20%, MCP-1 by 75% and GRO by 60% in human mesenchymal stem cells treated with TGFβ. CONCLUSIONS: These studies highlight the potential of PDC and PDA to allow effective wound healing while preventing excessive scar formation.

P.FS07

Pirfenidone Treatment Of Deep Partial-thickness Burns In C57bl/6 Mice

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Background- The high incidence of hypertrophic scar formation is one of the many challenges to treating deep partial-thickness burns. Treatment modalities for hypertrophic scars include laser therapy, pressure bandages, revision surgery and others, but are only marginally successful. For this reason, we evaluated prophylactic treatment of deep partial-thickness burns with an anti-fibrotic drug (pirfenidone) in C57BL/6 mice. Pirfenidone is an FDA-approved anti-fibrotic drug for systemic use in the treatment of idiopathic pulmonary fibrosis and other fibrotic disorders. Methods- Deep partial-thickness burns were induced in anesthetized mice with scalding water. Experimental groups (n=6/group), were treated twice during the inflammatory phase of wound healing with one of 4 topical ointment formulations containing different drug doses. Skin biopsies were taken at the inflammatory, proliferative and remodeling phases of wound healing and assessed for pirfenidone's effect on αSMA expression, hydroxyproline deposition and reepithelialization. Results- Compared to vehicle ointment, 6.5% pirfenidone significantly reduced expression of αSMA 12 days after the induction of burns and modestly reduced hydroxyproline in the burn area 22 days after the induction of burns. Additionally, we determined that pirfenidone did not affect reepithelialization when mice were treated during the inflammatory stage of wound healing. Conclusion- The results from pirfenidone prophylactic treatment on mice with deep partial-thickness burns warrant further studies to validate its effectiveness in preventing hypertrophic scarring. Disclaimer- The opinions or assertions contained herein are the private views of the author and are not to be construed as official or as reflecting the views of the Department of the Army or the Department of Defense.

P.FS08

Topical Delivery Of A Focal Adhesion Kinase Inhibitor Results In Accelerated Wound Healing With Reduced Scarring in A Porcine Wound Model

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Background: Focal adhesion kinase (FAK) plays a pivotal role in transducing mechanical signals to elicit scar formation during wound repair. Clinical translation of FAK inhibitor (FAKI) therapy has been challenging, however, due to the lack of an effective drug delivery system especially for extensive burn and blast injuries and large excisional wounds. In a previous mouse study, pullulan-collagen hydrogel-mediated FAKI delivery to excisional and burn wounds accelerated healing and reduced scar formation. Here we evaluated the efficacy and safety of topical FAKI hydrogel therapy in a large animal model.

Methods: Four female Duroc swine were used to create multiple 25cm^2 deep partial-thickness wounds. With multiple excisions, each wound depth was uniformly set to be 0.070 in. to create near full-thickness in the center and deep partial-thickness in the periphery. Animals received either standard dressings or FAKI-releasing hydrogels immediately after wounding, and dressings and hydrogels were changed every 2-3 days until wounds closed and then every 4 days thereafter. Wound closure rate and scar formation were evaluated over 90 days.

Results: Porcine excisional wound healing was accelerated with FAKI hydrogel treatment. Wounds treated with FAKI hydrogel closed significantly faster on day 14±2.0 vs. day 24±2.4 for control wounds (N=4 for each group, statistical difference at $p<0.01$). Visually inspected scars at Day 90 were substantially reduced with FAKI treatment and histological analysis demonstrated that fibrosis was substantially attenuated.

Conclusions: Biomaterial-based topical delivery of FAKI was effective in improving wound healing and reducing scar formation in a large animal model, and holds great potential as a novel therapeutic strategy for wound and scar management of large and deep dermal wounds.

P.FS09

Determination Of Trfs And Tirnas Levels In Human Keloids

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Background: Small non-coding RNAs (ncRNAs) have been found processing various functions in human health and diseases while sequencing studies have suggested that transfer-RNAs (tRNAs) may serve as a major source of ncRNAs. tRNA-derived ncRNAs can be broadly classified into two groups: tRNA halves (tiRNAs) and tRNA-derived fragments (tRFs). However, the role of tRFs and tiRNAs expression in human keloids remains unknown. Methods: In this research, five keloid tissue samples and five normal skin (NS) samples were recruited to go through high-throughput sequencing study. The tRFs and tiRNAs expression levels were measured and normalized as tag counts per million of total aligned tRNA reads. Results: Venn diagram indicated 2660 genes expressed in both of the groups and 592 specific expressed genes in keloids. Pie plots were used to show the unique tRFs and tiRNAs of expressed level percentage of each subtype. tiRNA-5 and i-tRFs are major subtypes of tRFs/tiRNAs in most of the samples. 68 up-regulated genes and 5 down-regulated genes having fold changes ≥ 2 and p-values ≤ 0.05 are selected as the significantly differentially expressed genes by differential expression analysis of tRFs and tiRNAs. Conclusions: These results demonstrate a differential expression of tRFs/tiRNAs in human keloids as well as propel our understanding of this still vastly uncharted genomic territory. Further investigation on tRFs/tiRNAs with potentially important biological roles may provide new means for keloid mechanisms exploration and treatment.

P.FS10

Human-like Hypertrophic Scars In Loose Skinned Animals Via Dynamic Circumferential Mechanical Tension

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Introduction: Scar formation is the typical response to cutaneous injury in humans, but not loose-skinned animals. Human hypertrophic scars have significant levels of poorly organized collagen, loss of hair follicles, a raised appearance, and long-lived myofibroblasts. The only current method for producing human-like hypertrophic scars in loose-skinned animals requires the use of a palatal expander to add mechanical tension to an incisional wound. We hypothesize that using compression springs to apply dynamic circumferential mechanical tension to excisional wounds will result in human-like hypertrophic scarring. Methods: Compression springs were engineered to produce a range (1.5-9N) of forces. To apply circumferential mechanical tension on an excisional wound, the spring is first compressed by an internal suture and attached to the edges of a 6mm wound on the dorsum of mice with 6 simple interrupted sutures at 6 equidistant positions. The spring is then uncompressed by releasing the internal suture at day 3-5 post-wounding and removed 19 days post-wounding. Scars were photographed, fixed, and paraffin embedded at 19 days, 6 and 15 weeks post-wounding. Tissues were trichrome stained or immunostained for smooth muscle alpha actin (SMαA). Results: Springs below 4.5 N became distorted due to wound forces; therefore we used 9 N of force. An increase in SMαA positive myofibroblasts, scar area, absence of hair follicles, and an observable raised scar was observed 19 days post-wounding in mechanically loaded wounds compared to splinted wounds. At 6 weeks scar area and an observable raised scar were observed. 15 week post-wounding an observable raised scars was also observed. Determination of SMαA positive myofibroblasts and scar area for 6 and 15 weeks is ongoing. Conclusion: We report the use of compression springs to produce human-like hypertrophic scars in loose-skinned animals via mechanical tension. This method is valuable due to being inexpensive and reproducible. We anticipate this model to be useful for testing agents for the prevention of pathological scarring.

P.FS11

Computational Analysis Identifies Putative Prognostic Biomarkers Of Pathological Scarring In Traumatic Wounds

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Pathological scarring in wounds is a common medical problem with limited prognostic options. In this study, we present an approach to identify wound proteins that could serve as putative prognostic biomarkers of pathological scarring in traumatic skin wounds. We developed and validated a computational model that predicts temporal changes in the levels of 6 cell types and 21 molecular mediators, including collagen, during an injury-initiated wound healing response. By analyzing thousands of model-simulated wound-healing scenarios, we identified proteins whose levels were consistently elevated (or decreased) in wounds that resulted in pathological scarring (i.e., hypertrophic scars or keloids). We found that interleukin(IL)-10, tissue inhibitor of matrix metalloproteinase (TIMP)-1, and fibronectin levels are consistently elevated as early as 2 and 3 weeks post-wounding in wounds that resulted in pathological scarring. Next, we quantified the predictive accuracy of these three protein markers using receiver operating characteristic curve analysis. We found that, using the levels of IL-10, TIMP-1, and fibronectin at 2 weeks post-wounding as predictors, we could predict the occurrence of a pathological scarring outcome at 6 weeks post-wounding with an 80% accuracy. Clinical validation of these model-predicted putative biomarkers could provide prognostic tools for objective clinical assessments of traumatic wounds. DISCLAIMER: The opinions and assertions contained herein are private views of the authors and are not to be construed as official or as reflecting the views of the U.S. Army or of the U.S. Department of Defense. This abstract has been approved for public release with unlimited distribution.

GROWTH FACTORS

P.GRO01

Protease Resistant Growth Factor Formulations For Healing Of Chronic Wounds

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Chronic wounds such as venous leg ulcers (VLU), pressure sores and diabetic foot ulcers are challenging clinical problems that affect a growing number of people due to the global expansion of the elderly, diabetic and obese. VLU affect approximately 1% of the adult US population, i.e., 2.3 million individuals. These wounds generally do not heal for over a month, can last years in severe cases and require advanced healing modalities, such as growth factors. However, the clinical results of using growth factors for treatment of chronic wounds have been very modest. Here, using chronic wound fluid from patients, we show that chronic wounds have high levels of proteases particularly neutrophil elastase. We report that neutrophil elastase degrades the clinically approved growth factor PDGF (platelet derived growth factor) in minutes, thereby rendering PDGF ineffective. We further report the development of novel PDGF formulations that extend the half-life of PDGF in the presence of neutrophil elastase. These formulations were tested in wound fluid derived from chronic wound patients (n=10), and we report that the PDGF was protected from degradation for up to 24 hours. Finally, we tested the formulation in a rodent chronic wound model with high neutrophil elastase and observed that the protected growth factor induced granulation and vascularization while the unprotected version displayed no effect (n=4 mice/group). Our results indicate that the developed PDGF formulation extends the life of the growth factor in chronic wounds by 24 hours. Therefore it is extremely promising for improving healing outcomes in chronic wounds.

P.GRO02

A New Mechanism Of Latent Tgf-b1 Presentation In Lung

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BACKGROUND: Accumulation of scar tissue in fibrosis diminishes organ function. Fibrosis is characterized by the chronic co-existence of macrophages (MΦ), which produce pro-fibrotic growth factors and myofibroblasts (MFs), which secrete and contract collagen. Our recent data show that MΦ express latent TGF-β1, which is only activated in co-culture with MFs upon direct contact. We investigate the mechanism how MΦ present latent TGF-β1 to MFs for local activation. Regulatory T cells are known to present latent TGF-β1 using the transmembrane protein glycoprotein A repetitions predominant (GARP).

METHODS: To test whether tissue MΦ express GARP, we immuno-colocalized GARP with MΦ marker CD68 in normal, inflamed and fibrotic human lung. Primary cultures of human MΦ were tested for GARP expression by Western blotting, flow cytometry and RT-PCR.

RESULTS: Our results show that GARP is expressed in MΦ of normal (n=17) and inflamed lung (n=32) and on the surface of *in vitro* polarized pro-inflammatory MΦ (6%). Western blots performed with cultured MΦ under non-reducing conditions detect GARP, LAP and TGF-β1 at 250 kDa, identifying the GARP-LAP-TGF-β1 complex. To test whether MFs release latent TGF-β1 from MΦ-GARP, we measured active TGF-β1 levels in MF co-cultures with GARP-expressing and -depleted MΦ. The release mechanism is currently under investigation.

CONCLUSIONS: Collectively, our results indicate that GARP serves as novel surface anchor on MΦ to provide a local TGF-β1 source in inflammation and fibrosis. Interfering with local TGF-β1 presentation and/or activation are potential novel anti-fibrotic strategies.

INFECTIONS & BIOFILMS

P.IB01

Human Cryopreserved Placental Tissues Inhibit Wound Associated Bacteria Biofilm Formation

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BACKGROUND: Biofilm, a community of bacteria, is tolerant to antimicrobial agents and host immune defenses due to their self-secreted protective extracellular polymeric substance. Biofilms are ubiquitous in chronic wounds, and the presence of biofilm correlates with non-healing. In wounds, biofilms reoccur within 24 hours post-debridement, therefore, prevention of biofilm formation is a desired property for wound care products. It has been shown that in a chronic DFU clinical trial that the use of a human cryopreserved viable amniotic membrane (CVAM) resulted in a high rate of wound closure and reduction of wound related infections. Our previous study demonstrated that CVAM possesses intrinsic antimicrobial activity against a spectrum of wound-associated bacteria under planktonic culture condition. METHODS: In this study, we evaluated the effect of CVAM and cryopreserved viable umbilical tissue (CVUT) on the biofilm formation of *S.aureus* and *P.aeruginosa*, the two most prominent pathogens associated with chronic wounds. The biofilm formation was measured using a high throughput method and an ex vivo porcine dermal tissue model. RESULTS: We demonstrated that the formation of biofilm on synthetic surfaces and porcine dermal tissue explants was significantly reduced in the presence of CVAM or CVUT-derived conditioned media compared to the control assay medium. CONCLUSIONS: This is the first study demonstrating that human cryopreserved viable placental tissues prevent biofilm formation. Anti-biofilm activity together with anti-inflammatory, anti-fibrotic and proangiogenic activities make placental tissue ideal for management of chronic wounds and should be considered as a desired biomaterial for engineering of new wound therapies.

P.IB02

Stem Tomography Of Hyper Biofilm Producing Persister Pseudomonas Aeruginosa

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Background- Persisters such as small colony variant (SCV) of *Pseudomonas aeruginosa* belong to the variant of regular bacteria, often biofilm associated, which show tremendous antibiotic tolerance in the host tissue. Superiority in SCV infection is often linked due to its complex biofilm structure. Thus we aimed to explore the structural organization of a persister variety of *Pseudomonas aeruginosa* biofilm. Methods- *P. aeruginosa* (PAO1) and its SCV Δ*wspF* were studied. Scanning transmission electron microscopy (STEM) and STEM tomography were used to explore the three dimensional structure of PAO1 and Δ*wspF* biofilm. We performed Energy Dispersive X-ray Spectroscopy (EDS) for elemental analysis of different biofilm layers of Δ*wspF*. Besides, scanning electron microscopy enabled visualization of extracellular polymeric substance, extracellular DNA (eDNA) and biofilm thickness. Results- Both PAO1 and Δ*wspF* displayed presence of thread like structure of eDNA (DNAse sensitive) and extracellular vesicles like structures as visualized through STEM tomography. Δ*wspF* was thicker than that of PAO1. Ultrastructure of Δ*wspF* biofilm revealed more electron scattered bacteria in apical layers, which appeared white in dark field STEM images. Bacteria at the surface-anchoring basal layer appeared dark. Contrast differences, as determined by EDS, between apical and basal layers of bacteria was attributed to differences in mass. Apical white bacteria had more mass and were heavier than the basal dark bacteria. Elemental analysis showed more abundance of nitrogen and oxygen in the apical layer. Thus the basal layers were hypoxic and metabolically silent. In both PAO1 and Δ*wspF* biofilm, bacteria were most compact at the base having abundance of cell debris. Conclusions- Insights into the ultrastructure of bacterial biofilm is likely to provide cue on their pathogenicity and should prove to be helpful in developing countermeasure strategies.

P.IB03

Genomic Responses Of Pseudomonas aeruginosa During Early And Biofilm Infection Of Wounds

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BACKGROUND: *Pseudomonas aeruginosa* is an opportunistic pathogen that frequently infects wounds, impairing their healing. We tested hypothesis that gene expression changes of *P. aeruginosa* as it adapts to the wound at early or late times (i.e., biofilm formation) are required to induce wound inflammation and impair healing, and that knocking out the genes that greatly change expression can cripple the bacterium's pathogenicity. METHODS: To evaluate gene expression changes during infection, we inoculated *P. aeruginosa* into full-thickness dermal-excision wounds (rabbit ear model), and then used RNA-sequencing to generate the bacterium's transcriptome profiles. To study global gene expression changes at early times of infection, the full-thickness wounds were infected on post-wounding day 3 with planktonic *P. aeruginosa* (10^6 CFU), and the bacteria were then harvested after 0, 2, 6, and 24 hrs. To study late-stage infection, the wounds were treated with topical antibiotics at 24 hrs post-infection, to kill planktonic cells, which resulted in biofilm-predominant infection; then the bacteria were harvested after 5 and 9 days RESULTS: During early infection, *Pseudomonas* up-regulated genes enriched most into Clusters of Orthologous Groups (COGs) categories 'cell motility', 'inorganic ion transport and metabolism', and 'secretion and vesicular transport'. In contrast, during late-stage infection, three COG categories stood out as being most up-regulated: 'defense mechanisms', 'secondary metabolites biosynthesis', and 'carbohydrate transport and catabolism'. Our transcriptomes of *Pseudomonas* infecting rabbit excision-wounds were also compared with previously published transcriptomes of *Pseudomonas* infecting the wounds of other species. CONCLUSIONS: Our *P.aeruginosa* gene expression data were more like that of human burn wounds than of that of mouse burn or excision wounds. Our ongoing studies use gene-knockouts to test for specific *P. aeruginosa* genes required for wound pathogenicity.

P.IB04

Pseudomonas Aeruginosa Biofilm Infection Of Partial-thickness Burn Wounds In Sprague-dawley Rats

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Background: Over 400 soldiers evacuated from combat with craniomaxillofacial injuries suffered from burns from 2001-2011. *Pseudomonas aeruginosa* infection is the highest concern in burn care, due to its virulence, antimicrobial resistance, and propensity to form biofilms. Methods: In this study, we characterized *P. aeruginosa* biofilm formation in a deep partial-thickness burn wound model in rats. Deep partial-thickness burn wounds, ~10% of the total body surface area, were created in anesthetized male Sprague-Dawley rats (350-450g; n=84) using a modified Walker-Mason scald model. Immediately post-burn, 100µl of a clinical strain of *P. aeruginosa,* strain 1244 ($1x10^3$, $1x10^4$, or $1x10^5$ cells/wound) in PBS was spread over the burn surface. At 1, 3, 7, and 11 days post-burning, animals were euthanized and tissue was collected for CFU counts, biofilm gene expression, and scanning electron microscopy (SEM). Results: *P. aeruginosa* developed robust wound infections, plateauing at ~$1x10^9$CFU/g burn tissue within 7 days. Expression of alginate genes and other biofilm genes indicated formation of a mature *P. aeruginosa* biofilm within the burn. Additionally, SEM showed *P. aeruginosa* invaded 200-300µm into the burn eschar. Conclusion: The development of *P. aeruginosa* biofilms within deep partial-thickness burn wounds has not been demonstrated experimentally until now. This model is valuable for testing anti-biofilm agents *in vivo* to advance burn care. Disclaimer: The opinions or assertions contained herein are the private views of the author and not to be construed as official or as reflecting the views of the Department of the Army or the Department of Defense.

P.IB05

Characterization Of The Host Response To Partial-thickness Burn Wounds Following Pseudomonas Aeruginosa Infection In A Rat Burn Model

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Background: Bacterial infection accounts for 75% of fatalities in patients with a burn greater than 40% of their total body surface area (TBSA). In this study, we characterized the host response to *Pseudomonas aeruginosa* biofilm infection within deep partial-thickness burn wounds using a scald rat burn model. Methods: Deep partial-thickness burn wounds (~10% TBSA) were created in anesthetized male Sprague-Dawley rats using a modified Walker-Mason scald model (n=84). Immediately post-burn, the wound was inoculated with a clinical strain (strain 1244) of *P. aeruginosa* ($1x10^3$, $1x10^4$, and $1x10^5$ cells/wound). At 1, 3, 7, and 11 days post-burning, animals were euthanized and samples collected for blood and serum analysis, histology, and myeloperoxidase activity of the burn wound. Results: *P. aeruginosa* developed robust infections within the burn wound, plateauing at ~$1x10^9$ CFU/g of burn tissue within 7 days. Blood analysis revealed elevated monocytes and neutrophils, coupled with corresponding pro-inflammatory cytokines in the serum. H&E staining of the burn eschar showed massive influx of inflammatory cells into the burn wound, correlating with inoculum. Myeloperoxidase activity was significantly elevated in inoculated groups by day 11. Conclusions: A dynamic host response was seen in a novel model of *P. aeruginosa* biofilm infection of deep partial-thickness burns. These studies may provide insights into improved treatment methods for burn care. Disclaimer: The opinions or assertions contained herein are the private views of the author and not to be construed as official or as reflecting the views of the Department of the Army or the Department of Defense.

P.IB06

Organo-Selenium Coated Polyester Bandage Inhibits Biofilms

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Background: Bacteria can form a biofilm in the dressing of a wound. This allows the bacteria to continually reseed the wound and overwhelm the host defense. This study is on the use of a dressing material that blocks biofilm formation in the dressing. Methods: A dressing was prepared that contained an organo-selenium (OS) coating which can catalyze superoxide formation in the dressing. This dressing was placed upon a wound on the back of a mouse and then different bacteria were injected below the dressing. *Staphylococcus aureus* and *Pseudomonas aeruginosa* were tested. After five days of growth, the amounts of bacteria growing in the bandage and in the wound were determined by a colony forming unit (CFU) assay. The amount of biofilm in the bandage was also determined by Confocal Laser Scanning Microscopy (CLSM). Results: Using colony forming unit (CFU) assays, over 7 logs of inhibition (100%) was found for both of the bacterial strains on the material of the OS coated wound dressing when compared with the control (untreated) dressing. CLSM along with IVIS Spectrum *In Vivo* Imaging were used to confirm the CFU results. What was surprising was that in the tissue under the OS dressing, the results of the CFU assay again showed a 7-8 log (100%)reduction in bacteria, versus that of the control covered wound. All experiments were done at least in triplicate. Conclusion: The OS dressing inhibits biofilm formation in both the bandage and in the wound, while control dressing showed biofilm in the bandage and the wound. Since the organo-selenium coating does not leave the bandage, this implies that a bandage can serve as a reservoir for biofilm formation in a wound. Thus, a bandage that does not inhibit biofilm formation in the bandage, makes it harder for the wound to resist biofilm formation.

P.IB07

ChloraPrep Use Alters Skin Microbiome Composition

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Background This study sought to understand how ChloraPrep induced changes in the skin microbiome may be related to surgical site infections. Methods Surgical site skin swabs were collected from consenting women 18 years or older undergoing breast reconstructive surgery over a 2-month period at Yale New Haven Hospital. Swabs were obtained immediately prior to and approximately 1 minute after skin sterilization with ChloraPrep. DNA was extracted using the Invitrogen Purelink Genomic DNA kit. PCR amplification of bacterial DNA was done using primers targeted to the 16S V1-V3 hypervariable regions. Genomic sequencing was performed with the Illumina MiSeq platform. A Wilcoxon Signed-Rank test was used to compare pre- and post-surgical preparation skin microbiome composition. Results 36 samples (18 pre-prep and 18 post-prep) from 9 patients were sequenced. On average, 20 operational taxonomic units (SD±7.4) were found within each sample. In each pre-prep and post-prep sample, Streptococcus species comprised an average of 1.5% (SD±0.1) and 3.7% (SD±3.8) of the skin microbiome, respectively, and Enterobacteriaceae genera comprised an average of 4.2% (SD±5.7) and 14.1% (SD±12.6) of the skin microbiome, respectively. Post-prep proportions were statistically significantly higher than pre-prep proportions for Streptococcus species ($S= 56$, $p=0.0063$) and Enterobacteriaceae genera ($S=79.5$, $p<0.0001$). There was no statistically significant pre- and post-prep difference in the proportion of Staphylococcus and Corynebacterium species, which are also known to be components of normal skin flora. Conclusions ChloraPrep appears to have differential effects on certain microorganisms, leading to an increase in the proportion of the overall skin microbiome comprised by Streptococcus species and Enterobacteriaceae genera, both of which have been implicated in surgical site infections. Appropriate perioperative antibiotic dosing should be employed to potentially decrease the risk of such infections.

P.IB08

Multidisciplinary Wound Infection Guideline Opportunities

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Wound infections increase patient, economic and clinical burdens of care. International consolidated wound infection guideline (ICWIG) developers identified and content validated evidence-based ICWIG recommendations to harmonize multidisciplinary team practice, encourage timely referral and improve the consistency of care across specialties and settings. Purpose: Describe needs for wound infection education and research based on content validity and standardized levels of evidence supporting ICWIG recommendations. Methods: Nineteen volunteer wound experts used structured literature searches of PUBMED, Cochrane and CINAHL literature from 1986 through 2013 to develop evidence-based wound infection recommendations. Forty-two independent wound expert respondents to an online survey used judgment quantification to rate each recommendation as relevant (3-4 on a 1-4 rating scale) or conferring more benefit than harm (1 on a 0-1 rating scale). A recommendation was content validated as relevant if at least 75% of respondents rated it 3-4. Results: Most (88.8%) recommendations were rated relevant and beneficial to patients (summarized in checklist format). Twenty recommendations were rated irrelevant and harmful by most respondents. Recommendations with high content validity and low evidence (requiring more research) or with low content validity and ample research (opportunities for education) are summarized. Conclusion: Relevant, safe recommendations inform team wound infection management across specialties and settings, while highlighting research and education opportunities.

P.IB09

Effective Non-cytotoxic Antibiofilm Dissolvable Dressing Powered By Ultralow Level Of Gallium And Silver

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BACKGROUND: Annual cost of treatment of chronic and burn wounds exceeds $25 billion in the U.S. Biofilms are a key factor in delayed healing in chronic wounds. Here we show that antibiofilm gallium (Ga^{3+}) and antimicrobial silver (Ag^{+}) ions work synergistically at non-cytotoxic concentrations to kill bacteria encased in biofilm. Ga^{3+} sensitizes bacteria in biofilm by substituting its Fe^{3+} uptake and Ag^{+} readily kills the sensitized bacteria.

METHODS: Biofilms containing 10^8 CFU of *P. aeruginosa* were established on a biologic dressing over 48h and rinsed with saline to remove planktonic bacteria. Moist biofilm samples were treated with dissolvable polymeric dressings which were fabricated as a composite of a 200 nm thick polyelectrolyte multilayer (PEM) nanofilm supported on a dissolvable 20 µm thick polyvinyl alcohol (PVA) layer. PVA layer dissolved leaving the nanofilm in intimate contact with biofilm where it released Ga^{3+} and Ag^{+} ions in a sustained manner in microenvironment of the biofilm.

RESULTS: 4Log_{10} CFU reduction and >90% biofilm mass dispersal was achieved from non-cytotoxic concentration of Ga^{3+} (4 µg/cm^2) and Ag^{+} (10 µg/cm^2), when these ions were delivered through a 200 nm thick PEM nanofilm. In contrast, only 1 Log_{10} CFU reduction was seen when (a) Ga^{3+} was incorporated in the PVA layer even at a level 20x of that in nanofilm, or (b) Ga^{3+} and Ag^{+} were formulated as topical solutions. This demonstrates that simultaneous and sustained release of Ga^{3+} and Ag^{+} ions in intimate contact with biofilm is required for effective antibiofilm activity. Furthermore, commercial silver-based antimicrobial dressings were not only ineffective against biofilm but also cytotoxic per MTT cytotoxicity assay.

CONCLUSIONS: Pairing of silver and gallium in a PEM nanofilm is suitable for assessment in the treatment of biofilms in chronic wounds.

INFLAMMATION & IMMUNITY

P.II01

Determining The Mechanism Of Action Of Keratin & Kap Biomaterials

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The purpose of this study was to identify the mechanism of action (MOA) of ProgenaMartix™ which is composed of keratin and keratin associated protein (KAP) matrices in accelerating wound healing in chronic and acute wounds. Preliminary evidence in diabetic mice and acute wounds in porcine studies indicate that the wounds respond well immediately with these advanced tissue matrices but the mechanism of action (MOA) is poorly understood**.** We measured human neutrophil elastase activity *in vitro* by fluorescence resonance energy transfer (FRET) assays and *in vivo* using a commercially available point of care diagnostic to determine the elevated levels of host proteases. We also measured cytokine levels in the porcine model and neutrophil cell viability and the mobilization of macrophages in a DB mouse model system. Our findings indicate the KAPs in ProgenaMatrix™ are actively suppressing the protease activity of neutrophil elastase which is concomitant with the reduction of periwound redness. Furthermore, ProgenaMatrix™ enriched in KAPs seems to dramatically improve the viability of neutrophils and enhances the mobilization of type I and II macrophages.

P.II02

Diabetes Alters Macrophage Polarization Resulting In Persistent Inflammatory Response

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BACKGROUND Macrophages exhibit functional heterogeneity and differentiate (M1 or M2 polarization) as a result of their microenvironment. The M1 phenotype is characterized by a pro-inflammatory state. The M2 phenotype is characterized by anti-imflammatory functions including tissue remodeling. Diabetic wounds demonstrate poor wound healing due to a variety of reasons, including dysregulated inflammation. We hypothesize that prolonged polarization of macrophages towards a M1 phenotype is involved in the persistent inflammatory response and poor wound healing seen in diabetic wounds.

METHODS A murine in-vitro macrophage model was used in this study. RAW 264.7 as well as murine bone marrow derived macrophages (BMM) were grown in cell culture, were treated with IFN/LPS or IL-4 $_{to}$ induce polarization (in RAW cells), and were harvested for PCR and Reactive Oxygen Species (ROS) Analysis. Statistical comparisons were made using Student's t-test, with a $p<0.05$ considered statistically significant. RESULTS In the RAW cell model, cells stimulated with IFN and LPS demonstrated a M1 phenotype with increased expression of TNF, IL-6, and STAT-1 ($p<0.05$). Cells stimulated with IL-4 showed increased expression of M2 phenotypic markers such as MRC and IRF4 ($p<0.05$). The IFN/LPS stimulated cells also exhibited increased ROS production ($p<0.05$). Using this information for characterization of the macrophage phenotypes, BMM from heterozygote and diabetic mice were examined. Diabetic macrophages demonstrated increased expression of IL-6 and decreased expression of MRC, IRF4, and STAT-6 compared to heterozygote macrophages, suggestive of a M1 phenotype. CONCLUSIONS Diabetic macrophages demonstrate a proinflammatory (M1) phenotype compared to non-diabetic macrophages which demonstrate a regenerative (M2) phenotype. This likely contributes to the poor wound healing noted in diabetic wounds and offers a future therapeutic target.

NOVEL THERAPIES

P. NOV01

Improved Wound Healing In Swine Model With Cerium Oxide Nanoparticle Conjucated With Microrna 146a

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BACKGROUND Our lab has previously shown that local delivery using cerium oxide nanoparticles conjugated to miR-146a (CNP-miR146a) improves wound healing in a murine model through decreased inflammation and improved angiogenesis. The purpose of this study is to evaluate the effect of CNP-miR146a on wound closure in diabetic pig. METHODS Diabetes was induced in a domestic white pig with a single dose of streptozotocin and confirmed with blood glucose measurements 4 weeks after administration. Two sets of five full thickness excisional wounds (1inX1in) were created on the back of pigs with a depth of approximately 2cm to reach the subcutaneous fat in all wounds. Half the wounds were treated with CNP-miR146a while the other half were treated with PBS (control) only. All wounds were digitally photographed in the presence of a standard reference ruler. Wound area was calculated using the ImageJ software. RESULTS Five of ten wounds on the right side were treated with CNP-miR146a at day 0 as described. Five wounds were treated with PBS as control. Digital imaging of wound was performed on day 0, 3, 7, 10, 14 for measurement of wound area. The wound area as measured by digital planimetry was plotted graphically. Data expressed as Mean in square centimeters. CONCLUSIONS Our results indicate accelerated wound healing of CNP-miR146a treated diabetic pig wounds at day 10 and 14 post healing based on decreased wound size. This porcine model better represents human diabetic wounds and provides support for CNP-miR146a as a possible therapeutic to improve wound healing.

P. NOV02

A Novel Non-contact Electric Field Therapy Enhances Angiogenesis And Wound Healing In Porcine Model

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Electric field (EF) stimulation of tissue repair is a promising strategy for treating non-healing diabetic ulcers, but progress in developing EF-based therapies is hindered by a poor mechanistic understanding of the EF interactions with cells and tissues. Our recent theoretical and experimental studies reported discovery of a novel EF modality for non-contact stimulation of vascular cells that results in activation of angiogenic signaling under normal and diabetic conditions. This study tests the hypothesis that the novel wireless electrotherapy based on the recently discovered EF modality will improve wound healing in the porcine model, resulting in enhanced vascularization and faster healing, as compared to non-stimulated wounds. Full-thickness wounds (n=16/animal) were created on the dorsal side of adult Yorkshire pigs (n=3 animals) and filled with saline or hydrogel (n=8/group). Half of the wounds were treated using a custom set-up that delivered non-thermal, non-contact EF therapy for 1 hr/day, 5 days/wk, for 2 weeks. Effects of EF, including wound closure, morphology, granulation tissue area, wound vascularization and collagen deposition were quantified using blind assessment of wound images or paraffin-embedded and stained tissue sections. EF therapy resulted in improved healing phenotype, significantly enhanced wound re-epithelization at day 5 and increased granulation tissue formation and vascularization, with evidence of improved collagen organization and a reduced fibrosis, as compared to no-EF controls. Studies are ongoing to quantify wound strength and inflammation. In conclusion, these results demonstrate that the novel EF-based technology is promising for regenerative wound healing therapies to help accelerate closure of chronic wounds and decrease amputations in patients with diabetic and other chronic ulcers.

P. NOV03

A Novel, Sterlized Microvascular Tissue Product Improves Healing In A Murine Pressure Ulcer Model

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Background: Processed microvascular tissue (PMVT) human structural allograft is derived from lyophilized human tissue containing microcirculatory cellular components. Since PMVT serves as a source of ECM, growth factors, cytokines, and chemokines modulating angiogenesis, inflammation, apoptosis, and endogenous cell recruitment, we hypothesized its application will accelerate wound regeneration in a validated pressure ulcer (PU) model developed in C57BL/6 mice using two 24hr cycles of skin ischemia/reperfusion created by placement and removal of external magnets. Methods: Two identical PU injuries (n=50 female mice) were treated with (a) topical particulate PMVT, (b) injected rehydrated PMVT, or (c) saline control injection, and assessed daily for closure rates, scab formation/removal, and temperature. A baseline control cohort (n=5) was euthanized at day 0 and treatment group cohorts (n=5) were sacrificed at 3, 7, or 14 days post-injury. The PU injuries were collagenase-digested for flow cytometric analysis of inflammatory, reparative, and stem cell frequencies; and analyzed by H&E histology and immunofluorescence. Results: PVMT accelerated wound closure; most notably, topical PMVT significantly increased mean closure from d5 (13% vs. -9%) through d13 (92% vs. 38%) compared to PBS controls ($p<0.05$). PMVT also hastened scab formation/removal; significantly accelerated disappearance of inflammatory myeloid (CD11b+) cells while upregulating α-smooth muscle actin, VEGF-A, and PLGF; and raised skin temperature surrounding the PU site, consistent with increased blood flow. Conclusions: These results indicate that PMVT has potential as an advanced treatment for restoring normal tissue function in ischemic wounds and merits clinical study.

P. NOV04

Fl2 siRNA In A Concentrated Poloxamer Platform For Acute And Burn Wound Healing

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Microtubules (MT) are intracellular polymers that provide the cell structural rigidity and act as important regulators of many cellular processes, including cell migration. The dynamicity and function of the MT cytoskeleton are determined in large part by its regulatory proteins, including the recently discovered enzyme Fidgetin-like 2 (FL2). FL2 severs and depolymerizes microtubules specifically at the leading edge of polarized cells. The localized nature of FL2 activity enables it to significantly impact specific cellular functions, namely cell migration. *In vitro,* down-regulation of FL2 expression with siRNA results in a more than two-fold increase in cell migration rate, with a noted enhancement of directional movement. To investigate these findings *in vivo,* we utilized a platform consisting of concentrated poloxamer (CP) (PluroGel) to deliver collagen microparticles containing siRNA to the site of injury. Topical application of FL2-CP-siRNA to murine animal models of full-thickness excision wounds and full-thickness burn wounds resulted in significant improvements in both the clinical and histological characteristics of the wound zone compared to controls. Wound healing occurred more rapidly and with high fidelity, resulting in properly organized collagen substructure and decreased scarring. Taken together, these findings indicate that FL2 is a promising target for continued therapeutic development in wound healing and that concentrated poloxamers offer a promising delivery platform.

P. NOV05

New Wound Classification Method: Direct Coding System From Korea

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Background: Various guidelines for wound assessment have been suggested but none of them are widely accepted as a standardized method for various wounds. Therefore, there is a need for developing a standardized and practical wound assessment tool, which is easily applicable to a variety of wounds. Here, we propose a D.I.R.E.C.T. coding system to guide healthcare professionals involved in wound care as a simple but efficient wound evaluation tool. Methods: Eleven Korean specialists including plastic surgeons and wound, ostomy, and continence nurses participated in the development the D.I.R.E.C.T. coding system as a practical wound assessment tool and a guide to establish treatment regime. Results: The D.I.R.E.C.T. coding system classifies all types of wounds on the basis of the Debridement of necrosis, Infection control, Revascularization, Exudate control, Chronicity, and Top surface, which are abbreviated by the acronym 'D.I.R.E.C.T.' The system has several superior points compared to the other systems. First, the system is versatile and thus applicable to the wounds with various etiologies and occurring locations. Second, it provides detailed grading based on the wound status, enabling clinicians to track the healing progression or regression easily. Third, the system covers critical physiological points important for wound healing while the other systems focus on limited area. Finally, the theoretical basis of the system is easy and straightforward that makes its application user-friendly. Conclusions: As a practical wound assessment system, the D.I.R.E.C.T. coding will enable healthcare professionals responsible for wound care to grade and assess all kinds of wound with simplicity and systemicity. Because it is also very easy to learn and apply to real practice, we think it may be universal wound grading tool.

D.I.R.E.C.T. coding system	
D	Debridement
I	Ischemia
R	Revascularization
E	Exudate
C	Chronicity
T	Top surface

OXYGEN/HYPOXIA

P.OX01

Pigment based Differential Regulation Of Redox Homeostasis In Hypertrophic Scar

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BACKGROUND: Dyspigmented hypertrophic scar following full-thickness wounds and burn injury has a negative psychological impact on patients. Despite a thorough understanding of normal pigment synthesis cascades, the cellular and molecular mechanisms leading to this specific dyspigmentation type remain unknown. The multitude of cell types and molecules involved in wound healing, that simultaneously induce variations in pigment synthesis, exacerbate the difficulty of investigating this health problem. METHODS: Dyspigmented scars were produced in a red Duroc pig scar model. Differences in gene expression between hyper- and hypo-pigmented scar biopsy samples were investigated using genome-wide microarrays. RESULTS: Differences in the abundance of scores of transcripts clearly separated hyper- and hypopigmented scars in both principal component and hierarchical clustering analyses. The list of top regulated genes included many pleiotropic genes potentially affecting melanin synthesis directly or indirectly. Pathway enrichment analysis of significantly (p-value < 0.01, LEF > 1.3) differentially regulated genes in hyper- and hypo-pigmented scars identified 10 pathways more significantly present in hypo- relative to hyper-pigmented scars (p-value difference < 1 log and > 2x in hyper-pigmented). Six out of these pathways, namely, the mitochondrial dysfunction, arginine degradation I and VI, nitric oxide signaling in the cardiovascular system, urea cycle, and the citrulline biosynthesis are central to maintaining redox homeostasis. Genes underlying the identification of several of these pathways include the mitochondrial NADH dehydrogenase II (MT-ND2), arginase 1 (ARG1), and ornithine carbamoyltransferase (OTC). CONCLUSIONS: Results of this work identify mechanisms involved in dyspigmentation pathogenesis and provide target candidates for prophylactic treatment and therapy.

P.OX02

The Effect Of Fractionated Ablative Carbon Dixoxide Laser With Lidocaine Spray On The Survival Of Skin Flap In Rats

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Background: Topical lidocaine is a traditional local anesthetic which improves the survival of random pattern skin flap in rats. Fractionated ablative carbon dioxide laser was also introduced as a new drug delivery-enhancement technique. The potential effect of fractionated ablative carbon dioxide laser and topical lidocaine spray (Xylocaine) on random pattern skin flap survival was investigated.

Methods: Forty eight male 2-month-old Sprague-Dawley rats were randomly divided into four groups, a control group, lidocaine group, fractionated ablative carbon dioxide laser group, and fractionated ablative carbon dioxide laser + lidocaine group (n=12 in each group). In all groups, caudally based McFarlane type random-pattern skin flaps were elevated. On postoperative day 7, the areas of necrotic flap were measured and percentages of flap survival were calculated. The number of vessels and neutrophil count was evaluated. Anti-rat VEGF antibody and CD31 antibody activity were measured.

Results: The survival area of the flap in fractionated ablative carbon dioxide laser + lidocaine group was significantly higher than that in the other groups. Mean neutrophil count in fractionated ablative carbon dioxide laser + lidocaine group was lower significantly than that of other groups. No statistically significance were observed between the groups in terms of the number of vessels. Anti-rat VEGF antibody and CD31 antibody activity was significantly higher in fractionated ablative carbon dioxide laser + lidocaine group than that in the other groups.

Conclusions: This study showed the positive effects of fractionated ablative carbon dioxide laser in the enhancement of random-pattern skin flap survival in rats with lidocaine. Fractionated ablative carbon dioxide laser treatment with lidocaine spray can represent a new therapeutic approach to enhance flap viability.

REGENERATION

P.REG01

Direct Neurotization Of Decellularized Muscle Matrix Leads To Enhanced Muscle Regeneration And Neural Tissue Ingrowth

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Background: Volumetric muscle loss can result in devastating functional deficits. Decellularized extracellular matrix has been a promising candidate for muscle regeneration, however functionalization requires motor nerve innervation. We examined the myogenic and neurogenic effects through direct neurotization of decellularized muscle matrix. Methods: Bilateral 1 cm latissimus dorsi defects were surgically created in 12 Sprague-Dawley rats. 8 defects were left alone, 8 were implanted with decellularized muscle matrix, and 8 were implanted with matrix then neurotized. 3-5 peripheral motor nerves innervating the panniculus carnosus were transposed onto the ipsilateral decellularized implant based on anatomic feasibility. Tissue was harvested on post-operative days 90 and 180. Samples were assessed through gross appearance, immunohistochemistry, and western blot analysis for myogenesis, inflammation, neovascularity, and neural ingrowth. Results: Both neurotized and non-neurotized matrices appeared well integrated within the surrounding muscle, while the defect alone healed with a thin layer of fibrous connective tissue. Neurotized matrix demonstrated greater infiltration by mature myocytes compared to non-neurotized controls based on MHC staining. CD-31 staining revealed similar levels of microvascular networks within neurotized and non-neurotized matrices, both greater than defect alone. Cholinergic neural tissue ingrowth was only observed in neurotized matrix. Direct stimulation of the matrix implants with a peripheral nerve stimulator demonstrated contraction of both neurotized and non-neurotized matrices, however a difference could not be grossly appreciated. Conclusion: Direct neurotization of decellularized muscle matrix in a model of volumetric muscle loss leads to more robust myogenesis and neurogenesis within the implant as compared to non-neurotized matrix and defect alone. Further studies are needed to evaluate functional differences in more detail.

P.REG02

Muscle Injury and Regeneration with N-acetylcysteine

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The repair process of wounded tissue involves the coordinated activities of muscle precursor cells (MPCs) in response to local and systemic signals. Following tissue injury, the microenvironment, which is characterized by excessive production of reactive oxygen species (ROS), is attenuated. Here we studied the efficacy of an antioxidant, N-Acetyl-L-Cysteine (NAC), *in vitro* and *in vivo* to ameliorate the damage that results from trauma so that repair and regeneration of wounded muscle tissue is enhanced and functionality is improved. MPCs were isolated from rat tibialis anterior muscles and exposed to increasing concentrations of H_2O_2 in the presence or absence of NAC for the *in vitro* assays. MPC proliferation, differentiation, and fusion into myotubes was assessed by IncuCyte microscopy, live/dead viability assays, MTS proliferation assays, and myosin heavy chain immunohistochemistry. CellROX reagent was used to detect ROS. For *in vivo* assays, adult female Lewis rats were subjected to compartment syndrome injury by applying 120-140 mmHg compression for 3hrs. Half of the injured rats received NAC injected intramuscularly and the other half received equal volume of PBS at 24, 48, and 72hrs after injury. Myogenesis, angiogenesis, fibrosis, and function were determined using RT-PCR, western blot, Masson's trichrome stain, and contractile force of the TA muscle as an isometric force (Hz), respectively *In vitro*, NAC administration resulted in a decrease in oxidative stress levels that was associated with significant survival benefit during oxidative damage. When used *in vivo*, NAC administration also showed decrease in tissue fibrosis, increase in myogenesis, angiogenesis and muscle function. These results suggest that treatment of skeletal muscle injuries with antioxidants may be a viable option for the prevention of long-term fibrosis and scar formation and improvement of recovery of muscle function.

P.REG03

Growth Factor Containing Hyaluronic Acid Gel For Hair Follicle Transplantation

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Background. It has been shown that epidermal stem cells that reside in the hair follicle (HF) bulge regions are important for wound healing. During the healing process these cells start differentiating and migrating to the wound bed thus contributing to wound closure. We have recently shown that wound healing using dermal grafts that contain HFs is comparable to split-thickness skin grafts (STSGs). Our analysis suggested that CD34 positive cells in the skin appendages contributed to wound closure by differentiating into basal keratinocytes during wound healing. The purpose of this study was to investigate HF cell behavior in a growth factor containing hyaluronic acid (HA) gel, use the gel as a vehicle to transplant HFs into rat full-thickness wounds and demonstrate that epidermal stem cells located in HF bulge regions can contribute to wound re-epithelialization. Methods. HFs were harvested from the rats by pulling the hair out by the root. 1.5% HA gel with defined keratinocyte medium was formulated and harvested rat HFs were cultured in the gel. Cell viability and proliferation were measured at days 1, 3 and 5. In addition, HFs in the gel were transplanted into rat full-thickness wounds. Wound closure and re-epithelialization were followed over time macroscopically and histologically and compared to STSG (n=6/time point/group). Results. The results indicated that when cultured in the HA gel with growth factors, cells started migrating from the HF to the gel, stayed viable and proliferated over time. In addition, it was shown that HFs in HA gel contributed to wound closure and re-epithelialization when transplanted to full-thickness wounds. Keratinocyte positive staining and wound re-epithelialization was observed on days 5 and 10 after transplantation. Conclusions. It is concluded that HA gel is a good vehicle for HF transplantation and this technique could potentially provide an autologous noninvasive alternative for STSG. This study was funded by the Wound Healing Foundation (Sponsored by Medline Industries).

P.REG04

Decellularized Fetal Tissue Matrix Enhances In Situ Skeletal Muscle Regeneration

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Background: Fetal wounds have been demonstrated to heal in a regenerative manner, however fetal extracellular matrix has not been well studied. We looked at the use of decellularized fetal soft tissue matrix for skeletal muscle regeneration. Methods: Composite soft tissue was harvested from the trunk of New Zealand White rabbit fetuses on gestational day 24. Bilateral 1 cm latissimus dorsi defects were surgically created in 12 rats. One defect was implanted with fetal decellularized matrix while the other was left untreated. Tissue was harvested on post-operative days 30 and 60. Samples were evaluated through gross appearance, immunohistochemistry, and western blot analysis for myogenesis, inflammation, neovascularity, and neural ingrowth within the explanted matrices and defects alone. Results: Scanning electron microscopy and picrosirius red staining revealed that fetal matrix is composed of a loose network of type III collagen reticular fibers. At the time of tissue harvest, the fetal matrix appeared well integrated within the surrounding native muscle as compared to defect alone, which healed with a thin layer of fibrous connective tissue. Fetal matrix played host to robust cell infiltration without evidence of immune rejection. Myosin heavy chain staining demonstrated substantial ingrowth of myocytes into the fetal matrix compared to defect alone. A dense microvascular network was appreciated within the implanted matrices. Furthermore, neuronal antibody staining revealed a time-dependent ingrowth of neural tissue into the fetal matrices as compared to the defects alone, which showed no evidence of ingrowth. Conclusion: Decellularized fetal soft tissue matrix is a promising scaffold for muscle regeneration, demonstrating significant myocyte proliferation and supporting microvascular as well as neural ingrowth.

STEM CELLS

P.ST01

The Effect Of Human Bone Marrow Mesenchymal Stem Cell-conditioned Medium On Healing Of An Infected Wound Model In Diabetic Rats

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The aim of this study was to investigate the effect of human bone marrow mesenchymal stem cell-conditioned medium (hBM-MSC-CM) on the microbial flora and tensiometrical properties of an infected experimental model of type 1 diabetes mellitus (TIDM). TI DM rats were induced by streptozotocin (STZ) and held for thirty days. Under general anesthesia and aseptic conditions, one f*ull-thickness excision wound* was *made on the back* of each *rat*. Rats were divided into two groups. The experimental group (N=6) received hBM-MSCs-CM 4 times while the control group (N=6) received regular medium only. All wounds were infected with methicillin-resistant staphylococcal aureus (MRSA) immediately after surgery. On days 4, 7, and 15 microbiological examinations were performed. We counted the numbers of microbes per sample, as colony-forming units (CFUs). The animals were euthanized at day 15 and standardized rectangular skin specimens were extracted across each wound and the adjacent normal skin. The specimens were mounted in a material testing machine. Data were analyzed by student t-test. Our results showed that hBM-MSC-CM significantly increased tensiomerical properties of repaired wounds and significantly decreased colony-forming units (CFUs) compared to control wounds. In conclusion, application of hBM-MSC-CM accelerated wound healing process in an MRSA infected cutaneous wound model in TI DM rats, as demonstrated by the significant increase in tensiometrical properties, and antibacterial activity.

P.ST02

The Effects Of Combined Photobiomodulation And Human Bone Marrow Mesenchymal Stem Cell-conditioned Medium On Wound Healing In Diabetic Rats

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We investigate the effects of Photobiomodulation and human bone marrow(hBM)-MSC-CM alone or in combination on histological, and stereological parameters, and the quantitative real-time polymerase chain reaction(RT-PCR) for the analysis of bFGF, HIF-1alfa, and SDF-1 α in the repairing wound in a chemical induced type one diabetes mellitus (TIDM) in rats. hBM-MSCs were isolated, and expanded. hBM-MSC - CM(CM) was prepared by culturing hBM-MSCs . TIDM was induced in 24 rats. For all animals, two incisions were made on their backs. Rats were divided into four groups . First group were considered as the control group. The second group received LASER; the third group received CM two times, the fourth group received LASER+ CM. On days 4, 7, and 15 skin samples were extracted for histology and stereology, and RT-PCR analyses of bFGF, HIF-1 α, and SDF-1 α gene expression.We observed that in proximal and distal wounds all treatment groups showed significantly better stereological results compared to control group. And in the most cases results of CM+Laser group was significantly better than other treatment groups. In addition stereological parameters respond ositively to CM, Laser, and CM+Laser treatments at inflammation, proliferation, and remodeling phases of wound healing process. In the most cases results of RT-PCR in both CM +Laser, and Laser groups were significantly better than CM and control groups.

LATE BREAKING POSTERS

P.LB01

Epidermal Stem Cells Interact With Adipocytes To Promote Healing In Obesity Wound

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BACKGROUND Obesity is a prominent global health issue, but its impeded wound healing remain unresolved. As epidermal stem cells (EpSC) and adipocytes are essentials in the structural, metabolic and healing process of skin, their interactions may influence wound healing in the obesity.

METHODS A rat model of diet-induced obesity was established with groups of sham-injury, injury without transplantation (Inju) and injury plus EpSC transplantation, then a 6-mm diameter full-thickness excision was developed on the dorsal skin. 1×10^5 epidermal basal stem cells isolated from neonatal mice skin and suspended in 30 μl 1×PBS were injected subcutaneously into each wound in EpSC group, as equivalent 1×PBS was injected in sham and Inju groups. Skin wounds and serum samples were harvested at 1, 3, 7 and 14 days post injury, and subjected to histological investigations and colorimetric detections.

RESULTS At 1, 3 and 7 days post injury, promoted scar area, wound contraction, re-epithelialization, collagen deposition and adipocytes repopulation were found in EpSC group (P<0.05, *vs* Inju). Simultaneously, a "down then up" fluctuation of serum FABP5 and FABP4, two fatty acid binding protein subtypes secreted by epidermal cells and adipocytes, respectively, was investigated in EpSC group at 1 and 3 days post injury, while FABP5/FABP4 ratios in EpSC group were higher at 7 and 14 days post injury (P<0.05, *vs* sham or Inju). Moreover, altered glucose and lipid metabolism containing serum lactate/pyruvate ratios, NAD^+/NADH ratios, insulin, triglycerides and total cholesterol were identified.

CONCLUSIONS EpSC-promoted wound healing in the obesity associates with adipocytes, and the metabolic interaction will be a novel breakthrough for treating obesity wound.

P.LB02

Revascularized Vessel As A Recipient In Microvascular Reconstruction Of The Lower Extremity

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Background: The purpose of this study was to demonstrate the safety and reliability of combined preoperative angioplasty and free-flap transfer in patients with peripheral arterial occlusive disease (PAOD) by analyzing the surgical outcomes.

Methods: Between September 2011 and October 2015, the patients who had undergone lower-extremity angiography and subsequent free-flap transfer were retrospectively reviewed. Data collected included demographics, perioperative data, and postoperative outcomes. The cases were divided into two groups; one group with microanastomosis performed on revascularized artery by balloon angioplasty and the other group performed on native artery. Multiple logistic regression model using propensity score and linear regression were computed to determine the association between preoperative angioplasty and the surgical outcomes.

Results: A total of 62 lower limb reconstruction cases (19 angioplastied cases and 43 native cases) were included in the study. Complications occurred in 6 cases of the angioplastied group and in 11 cases of the control group. The overall limb salvage rate was 100 percent during the average follow-up of 35 months in the angioplastied group and that of non-angioplastied, control group was 97.7 percent during the average follow-up of 38 months. Preoperative angioplasty was not a significant predictor for increased complications and longer postoperative downtime in logistic and linear regression model, both in weighted and unweighted model. Conclusions: The combined approach of preoperative endovascular revascularization and free-flap transfer for limb salvage in PAOD patients can be performed safely and effectively with acceptable morbidity.

P.LB03

Antimicrobial Functionalization Of Ovine Forestomach Matrix With Ionic Silver

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BACKGROUND: The decellularized extracellular matrix biomaterial, Ovine Forestomach Matrix (OFM), is an established scaffold for use in wound healing and tissue repair indications. We have recently developed an antimicrobial variant of OFM containing ionic silver, termed OFM-Ag. This study sought to characterize the functional properties of OFM-Ag in relation to its use as an antimicrobial wound dressing material.

METHODS: The antimicrobial effectiveness spectrum and wear time of OFM-Ag toward 11 microbial species encompassing clinically relevant bacteria, yeast and mold was determined using ISO20743 methodology. The cell compatibility of OFM-Ag was assessed via MEM elution cytotoxicity assay utilizing mammalian fibroblasts. Silver content and silver elution profile of OFM-Ag was quantified by atomic absorption spectroscopy, while retention of the native collagen architecture and matrix integrity was assessed via differential scanning calorimetry and scanning electron microscopy.

RESULTS: OFM-Ag demonstrated >4 $\log_{10}$ reductions toward all species tested, indicating broad spectrum antimicrobial effectiveness. Furthermore, OFM-Ag retained this effectiveness over a period of 7-days, the maximum antimicrobial wear time period tested. OFM-Ag was determined to be non-toxic to mammalian cells. The dressing was characterized as containing 0.30% w/w silver and upon elution the silver remained bound to the matrix, retaining effective antimicrobial concentrations in the material over 7-days of elution. Silver functionalization had no detrimental effects toward the composition or integrity of the matrix, with calorimetric analysis showing preserved native collagen structure.

CONCLUSIONS: The present work indicates OFM-Ag retains the intact extracellular matrix properties of OFM desirable for wound healing while providing sustained and potent broad spectrum antimicrobial effectiveness.

P.LB04

Crosslinking Agents For The Development Of An Infected Chronic Wound Model

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Based on the established correlation of both wound duration and biofilm with wound chronicity, we set out to maintain and infect an acute wound in an attempt to develop a novel chronic wound model. Prior studies demonstrated a ~350% delay in healing of 5% Glutaraldehyde (GL) cross-linked wounds, which was correctable with surgical debridement. In the present study, we screened 3 organic cross-linkers for their effects on wound healing rate and bioburden. Twenty full-thickness 2cm dorsal wounds were created in two pigs. Eight wounds were treated with 5% GL and 8 were treated with D-ribose/3% hydrogen peroxide (R-HP). Eight wounds were treated with 1 g/mL N-(3-Dimethylaminopropyl)-N'-ethylcarbodiimide (EDC) and 8 were treated with 11mM Genipin (GN). Four untreated wounds on each pig served as untreated controls. All wounds were inoculated with 10^6 CFU/mL *Staphylococcus aureus, Pseudomonas aeruginosa* and *Fusibacterium*. Wounds were covered with non-adherent dressings and healing was measured at day 1, 4, 7, 11 & 13. Wounds were biopsied and selectively cultured on days 1, 4 & 13. EDC-treated and GL wounds showed the greatest delay in wound healing, followed by GN. Treatment with R-HP did not result in healing rates different from untreated wounds. EDC-treated and GL wounds also showed the greatest bacteria, with total bioburden and *Pseudomonas* reaching >8log at days 4 and 13, while *Staphylococcus* showed 7log at day 4 with reduction to 5log at day 13. These data support the hypothesis that crosslinking and inoculation of the acute wound shows promise as a stable infected chronic wound model that may be useful for studies of debridement and other therapies.

P.LB05

Treatment Of Recalcitrant Chronic Wounds With A Hyaluronic Acid Dressing And A Micelle Matrix Surfactant: A Pilot Study

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BACKGROUND Chronic wounds fail to heal when the overlapping phases of wound-healing become disarrayed, but many treatments only target a single phase. If wounds become recalcitrant, particularly diabetic ulcers, amputation rates and 5-year mortality escalate. We tested the use of a hyaluronic acid dressing after pre-application of a surfactant; both treatments are FDA-approved, but they have never been tested in combination. We aimed to investigate whether combination therapy targeting multiple phases of wound-healing could provide superior healing outcomes.

METHODS Combination dressing-surfactant therapy was administered once-weekly for 12 weeks to patients with wounds failing standard care and persisting ≥6 months. Volumetric analysis was performed to calculate interval changes in wound size (cm3) before, at initiation, and termination of therapy. Sizes pre- and post-combination therapy were compared by paired T-Test. Percent size reductions during standard care versus combination therapy were compared by Student's T-Test.

RESULTS Total 4 patients with 4 wounds: 2 diabetic ulcers, 2 venous ulcers. Wounds had average 1 year duration and had failed to heal under standard wound care regimens. Sizes were decreased post-combination therapy ($p<0.05$), and percent size reductions were greater ($p<0.05$) compared to the preceding 6-month standard care interval. No wounds closed completely. No adverse events occurred.

CONCLUSIONS In patients with recalcitrant chronic wounds, combination therapies targeting different phases of wound-healing may synergize to provide a novel stimulus to the wound-healing apparatus. This may explain the salutary effects observed in patients treated with surfactant targeting the external wound macroenvironment and a hyaluronic acid dressing targeting the internal wound microenvironment.

P.LB06

Pregnancy Improves Skin Wound Healing In Murine Model

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Wound healing is a complex regenerative process involving various cell types and also requires a series of growth factors and hormones that act in concert to restore the integrity of the injured tissue. Many studies have shown that exposure to factors present in the serum of young mice restores the regenerative potency of aged cells. One idea is that pregnancy could represent a unique biological model of a naturally-shared circulatory system between young and old. We investigated the wound healing process in pregnant and non-pregnant mice via a laceration injury model. The mice were sacrificed at 7 and 14 days after injury, and hematoxylin/eosin and immunofluorescence staining were performed. We observed that the granulation tissue and layers of regenerated epithelial cells in wounded area were thicker in pregnant mice than in non-pregnant mice. Results from immunostaining of smooth muscle actin and CD31 indicated the appearance of greater numbers of myofibroblasts and blood vessels in wounded area in pregnant mice than in non-pregnant mice. Our results suggest that pregnant mice show superior wound healing compared to non-pregnant mice, suggesting that circulating factors present during pregnancy may play an important role in wound healing by enhancing the function of progenitor cells in the skin and increasing angiogenesis. Identifying rejuvenating factors within blood circulation during pregnancy could have potential for the development of novel therapies for wound healing.

P.LB07

Notch Activator, Jagged1, Results In Increased Wound Closure In An Ex Vivo Murine Skin Wound Model

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Introduction: Suboptimal wound healing affects millions of patients annually. We are interested in novel strategies to enhance the wound healing process in diabetic patients. We have previously shown that inhibiting Notch inhibits wound healing. Based upon our previous work, we propose that upregulation of Notch would increase rates of wound healing. JAG1 is a known activator of Notch. Therefore, we hypothesized that applying topical JAG1 to ex vivo excisional wounds on the backs of mice would result in increased Notch activity, and thus an increased wound healing rate as compared to untreated wounds.

Methods: Skin biopsies from 12-week old, healthy mice, (1-cm^2 full-thickness) were cultured ex vivo. A 3-mm wound was created in the center of the skin biopsy. Topical application of JAG1 (10 nM) or vehicle (PBS) was applied daily. Digital photographs were taken and histological and protein analysis were performed. Wound area was calculated as a percent area of the original wound size. Statistical significance was defined as $p<0.05$ using the students' t-test.

Results: Partial to complete re-epithelialization was seen in the wounded tissues over the experimental period in both the control & JAG1 treated groups. The mouse skin treated with topical JAG1 had an increased rate of wound closure when compared to wounds treated with PBS

Conclusions: JAG1 increases the rate of re-epithelialization of cutaneous wounds in an ex vivo murine wound-healing model, indicating that Notch signaling plays a crucial role in wound healing in mice. Based upon our findings, further study of Notch in wound healing should be conducted which may then lead to better therapeutics for the wound healing process in patients.

P.LB08

Comparison of Noninvasive Skin Perfusion Techniques

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Introduction Objective measurement tools are of great value to provide reliable image of a variety of pathological conditions including burn, traumatic wounds, diabetes, aging and shock because they are associated with changes in local or systemic blood perfusion. The aim of this study was to compare various currently used techniques to assess their validity and reliability.
Hypothesis We hypothesized that there are no quantitative/qualitative differences between microcirculation measurement techniques, to differentiating ischemia.
Methods In a group of 20 young adult New Zealand White rabbits, we rendered one ear ischemic and local tissue perfusion was measured and compared using laser speckle contrast imaging (LSCI, Moor FLPI-2), tissue viability imager (Tivi8000micro), thermal imager (FLIR C3), tissue oxygenation monitor (MoorVMS-Oxy), transcutaneous pO_2 and pCO_2 (Radiometer TCM4), and several pulse oximeters (Bionet Vet and Ohmeda Biox 3740).
Result All the perfusion measurement showed clear perfusion differences between the ischemic and non-ischemic ears. LSCI has shown the most consistent result. Tivi8000 showed similar results but sometimes was affected by skin color changes. Thermal imager camera also showed good results but the sensitivity was not the best. Both MoorVMS-Oxy and TCM4 had good results but required longer time to measure. Pulse oximeters had some difficulty when the main artery was ligated.
Conclusion Our preliminary results show the most consistent measurement outcomes with the use of LSCI technique. A combination of two different methods can give us a better understanding of perfusion measurement.

P.LB09

Oxandrolone In Combination With Propranolol Decreases Fibrosis In Post-burn Hypertrophic Scar Fibroblasts

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Hypertrophic scars are debilitating and decrease burn patients' quality of life; surgical excision and laser ablation are the most effective therapies. Oxandrolone in combination with propranolol reduces post-burn scars by a still unknown mechanism. Here, we treated burn patient-derived hypertrophic scar fibroblasts for 7 and 14 days with propranolol, oxandrolone, or their combination, and assessed basal and TGF-β-induced fibrotic signaling via Western blot. Oxandrolone alone or in combination with propranolol decreased basal α-SMA protein expression by 7($p<0.05$) and 14 days ($p<0.001$). TGF-β-induced α-SMA protein expression was reduced by oxandrolone alone or in combination with propranolol at 7 ($p<0.05$) but not 14 days. Oxandrolone plus propranolol decreased basal collagen-I protein expression at 7 and 14 days ($p<0.05$). N-cadherin protein levels decreased 7 days post-treatment with oxandrolone or oxandrolone plus propranolol ($p<0.05$). Oxandrolone alone or in combination with propranolol decreased basal fibronectin protein expression at day 7 ($p<0.05$) and TGF-β-induced fibronectin expression at day 14($p<0.05$). Basal protein expression of vimentin and collagen III was not affected by any of these treatments, while TGF-β-induced protein expression of vimentin and collagen-III was decreased by propranolol at day 14 ($p<0.05$). CD44, Integrin B1, and B5 protein levels were not affected by any of these treatments, though basal HAS-2 protein levels were decreased by 7 and 14 days treatment with oxandrolone plus propranolol ($p<0.05$). Collectively, these data show that combined treatments that aim to modulate both the catecholamine and the cortisol surge post-burn are the most effective in reducing fibrotic phenotypes in burn patients hypertrophic scar fibroblasts.

P.LB10

Wireless Biosensor On Dressing For Rapid Measurements Of Wound Biomarkers

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BACKGROUND: Measurements of molecular markers in wound fluid can provide valuable information for assessing wound status and monitoring wound healing. Currently, molecular analysis of wound fluid requires laboratory analysis, which is laborious, time-consuming and expensive. While point-of-care tests for measuring wound biomarkers have been demonstrated, they only provide qualitative results or require invasive sample collection which can disturb the natural wound healing process. To address these limitations, we demonstrate a novel dressing-based wireless biosensor for rapid measurements of wound biomarkers.

METHODS: The sensor is comprised of interdigitated carbon sensing electrodes with a silver coil antenna, which are screen-printed onto dressing. Measurements are wirelessly read using an impedance analyzer with a transmitter antenna.

RESULTS: We first investigated the sensor's capability for wireless measurements by detecting uric acid spiked in simulated wound fluid (50% v/v serum and 50% v/v buffer) from 0 - 800 μM. A direct correlation between the uric acid concentration and the detection signal was observed with excellent linearity (R^2 of 0.994) and responsivity (6.25kHz/μM) over the tested concentration range. We also studied the influence of a silver-containing antimicrobial wound dressing (AQUACEL® Ag EXTRA™) on the sensor response by placing the dressing on top of the sensor and found that it did not impede its performance. Lastly, we performed measurements of uric acid spiked in clinical wound fluids which showed that our sensor can accurately detect wound biomarkers in a complex extracellular matrix.

CONCLUSIONS: This wireless dressing-based biosensor offers a non-invasive method for quantifying wound biomarkers *in situ*, which can aid clinicians in monitoring wound status and predicting the healing process.

P.LB11

Changes In Macrophage Phenotype In Hypertrophic Scarring Population

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Hypertrophic scarring(HS) is observed in more than 70% of the severe burn survivors. HS is mainly composed of myofibroblasts and macrophages that secrete a significant amount of extracellular matrix. Imbalance in the macrophage phenotype and function can lead aberrant repair, and eventually scar in different pathological conditions. We hypothesized that HS has different expression of macrophage phenotype compared to non-burned controls and macrophage phenotype controls scarring sequelae. Methods: Scar biopsies with greater than 30% total body surface area burns(n=12) were compared to the skin biopsies from non- a burned area from the burn patients. Tissue sections were stained with CD68 (M1marker) and CD206 (M2marker) and immunofluorescence was utilized to analyze the expression. While in-vitro THP-1 cells were stimulated with M1 and M2 phenotype then co-cultured with a scar and skin fibroblast to elucidate the interaction between two distinct types of cells. Results: Both scars and burn control skin samples stained for CD68 and there was no significant difference in the expression between the two groups. However, scar biopsies had a significantly higher expression of CD206 (M2 marker) compared to the burn controls skin biopsies ($p<0.05$). In-vitro studies showed that M2 induced macrophages significantly increases collagen-1A expression in scar and skin fibroblast compared to non-treated fibroblast or co-culture with M1 induced macrophages ($p<0.05$). Conclusion: M2 phenotype macrophage is associated with the production of anti-inflammatory but pro-fibrotic factors like TGF-b. Presence of significant amount of M2 macrophages in scar biopsies and increase in ECM component in skin and scar fibroblast following co-culture with M2 macrophage demonstrate that scar pathology has a different phenotype of macrophages expression.