Late Breaking Abstracts

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P.LB01 - UNDERSTANDING EUROPEAN BIOCIDAL PRODUCTS COMITTEE OPINION TOWARDS USE OF POLYHEXANIDE IN HUMAN HYGIENE PRODUCTS

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Burn injuries are globally responsible for about 5% of total mortality and the overall global annual cost was estimated around 500 billion US dollars. Fire, scald, electricity, and chemicals are the main causes of resulting wounds. Strong chemicals such as acid induced wounds either due to accidents or increased violence in developing countries against females are difficult to treat. The ability of adipose tissue-derived mesenchymal stem cells (ASCs) to repair acid inflicted skin burnt wounds has not been reported yet. This study addresses the role of Vitamin E preconditioning of ASCs in counteracting acidosis stress in vitro and repairing acid burnt skin wounds in vivo. Rat ASCs were treated with 100μM Vitamin E for 24 hours, subjected to in vitro acidosis stress and analyzed for viability, paracrine release and alteration of gene expression. The in vivo study involved cell transplantation in rat model of hydrochloric acid inflicted skin burnt wounds followed by analysis of wound closure, re-epithelialization, histology and alteration in gene expression(n=6 rats each group). Results showed that Vitamin E preconditioning resulted in significant increase in viability (shown by LDH release and cell senescence), paracrine release [of stromal cell derived factor 1 alpha (SDF-1α), VEGF and basic fibroblast growth factor (bFGF) proteins] and up-regulation of pro survival genes (Bcl-2, SDF-1α, and bFGF genes) in preconditioned ASCs in vitro. Vitamin E primed ASCs induced significantly rapid re-epithelialization along with expeditious wound closure and up-regulated expression of epidermal (CK-8, CK-18, E-Cadherin) and dermal (Vimentin) genes while down-regulation of pro-apoptotic gene (Bax-2) in vivo. Hence, this study shows that Vitamin E preconditioning enhances the therapeutic potential of ASCs to repair the chemically damaged skin.

P.LB02 - INFLAMMATORY HYPOXIA - A COMMON BARRIER TO OXYGEN DELIVERY TO TISSUES

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Purpose: Hypothesis generation. Chronically diseased tissues are characterized by inflammatory hypoxia (IH). Reversal of IH, through the administration of high doses of oxygen therapy (hyperbaric oxygen), will break this vicious cycle and may normalize tissue responses and repair. Methods: Previous work in humans will be reviewed to demonstrate that IH exits in a human model of acute, self-resolving inflammation; the tuberculin reaction (TR). The data (n =20; 5 weak, 15 strong TR) demonstrate that strong reactors develop tissue hypoxia accompanied by a paradoxical increase in microvascular hemoglobin-oxygen saturation and a reduction in oxygen extraction. The authors conclude that IH induces a type of “protective regulation” that is similar to what others have called “oxygen conformance, cytopathic hypoxia or dysoxia” to explain the apparent neglect of hypoxic tissues to increase the extraction of oxygen from hemoglobin. Yet, by providing 100% inspired oxygen (1 atmosphere) to individuals at specific time points (24, 48, 72, 96 hours) during the TR, oxygen consumption (VO2, ml O2/kg/min) is increased from 3.5 and plateaus at 6.0, suggesting adequate oxygenation. However, a trial of 100% oxygen (2 atmospheres) results in a marked further increase in VO2 from 4.5 to 12 which continues to increase at 96 hrs. IH does not induce tissue neglect of oxygen but rather demonstrates an oxygen deficit that can only be met through effective tissue reoxygenation. Conclusion: Early time points during dermal inflammation establishes a condition of IH that can only be effectively re-oxygenated by increasing the partial pressure of oxygen within tissues. Re-oxygenation of compromised tissues must occur before successful treatment of these diverse chronic diseases can be expected.

P.LB03 - DANCING WITH EPIDERMAL STEM CELLS AND ADIPOCYTES: A TALE OF TWO HEALING PARTNERS IN OBESITY WOUND

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Global obesity is becoming increasingly prominent, while the mechanisms for obesity-impeded wound healing remain unclear. As epidermal stem cells (EpiSCs) and adipocytes are vital components in both the structure and healing process of skin, we hypothesize that potential associations between them may influence wound healing in the obesity. We established a rat model of diet-induced obesity, setting groups of sham-injury, injury without cell transplantation (Injury) and injury plus EpiSCs transplantation. Then we developed a 6-mm diameter full-thickness excision on the dorsal skin of obese rats, and injected subcutaneously 1×105 epidermal basal stem cells isolated from neonatal mice skin into each excision site in EpiSCs group, while equivalent 1×PBS (pH7.2-7.4, 10 mM) was injected in sham and Injury groups. Histological investigations showed that from 1 to 7 days after injury, EpiSCs transplantation significantly decreased scar area, promoted wound contraction, and accelerated re-epithelialization ($P<0.05$), as compared with Injury group. Interestingly, immunohistochemistry staining suggested a profound repopulation of perilipin A-positive adipocytes in the wound bed of EpiSCs group ($P<0.05$). Moreover, sirius red and Masson's trichrome staining also proved an increased yet ordered collagen deposition ($P<0.05$) in EpiSCs group. It is concluded that EpiSCs can mobilize adipocytes and evoke subsequent rehabilitation, indicating a novel perspective for the research and care of obesity wound.

P.LB04 - FIDGETIN LIKE 2 ACCELERATES EXCISIONAL WOUND HEALING IN PIGS

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Microtubules 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 developed a nanoparticle (NP) platform which is able to encapsulate siRNA and deliver the payload to multiple layers of tissue. Topical application of FL2-NP-siRNA to murine and porcine animal models of full-thickness excision 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. Most notably, relative to controls, FL2 NP-siRNA treatment in porcine models showed a more than three-fold decrease in wound depth after just 7 days. These results are striking as there is a 78% concordance between results of wound healing studies performed in humans and pigs. Taken together, these findings indicate that FL2 is a promising target for continued therapeutic development in wound healing.

P.LB05 - EFFICACY OF CHITOSAN-BASED DRESSING FOR CONTROL OF BLEEDING IN EXCISIONAL WOUNDS

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Introduction: Excessive bleeding is a complication of wound debridement in patients receiving anticoagulant treatment. Chitosan is a linear, positively-charged polysaccharide that has potential as a hemostatic topical dressing. This study examined the hemostatic efficacy of the chitosan based Opticell dressing (Medline Industries, Chicago, Ill.) in heparinized rats with excisional wounds mimicking debridement. **Methods**:Three paired, 12 mm excisional wounds were created on the dorsum of 600 gm Sprague Dawley rats 2 hours after intraperitoneal injection of Heparin 800IU/kg. Opticell or gauze dressings were applied with 3 seconds of gentle pressure.

Results: Total Bleeding: The dressings were left in place until cessation of bleeding. Ten minutes was enough time for complete bleeding cessation in both groups. Gauze and Opticell were weighed before and after bleeding cessation with the difference representing blood loss. Total blood loss was 627±47mg/10min with the standard gauze, but: 247±47 mg/10 min with Opticell. (p=0.002 Mann-Whitney). N=6 wounds per group. Rate of Bleeding: Gauze and Opticell dressings were removed and instantly replaced with 3 seconds of gentle pressure every minute until bleeding cessation. The removed dressings were weighed before and after application. There was less bleeding in the Opticell group at minutes one, two and three. Gauze 183 ±40, 140±30, and 109±15mg/min vs Opticell 91±17, 54±8, and 57±11 mg/min. ANOVA, Tukey's test, $p<0.05$. N= 12 wounds per group.

Conclusion: Topical application of Opticell dressing with chitosan has hemostatic effects that could be a useful tool to control bleeding associated with wound debridement.

P.LB06 - TOPICAL APPLICATION OF MESENCHYMAL STEM CELL-DERIVED CONDITIONED MEDIA PREVENTS BURN PROGRESSION

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Burn progression is a phenomenon in which areas adjacent to deep partial thickness injuries convert to full thickness injuries in part due to limited perfusion. The conditioned media of mesenchymal stem cells (MSC) are rich in factors that promote healing. We hypothesized that topical application of mesenchymal stem cell-derived conditioned media would prevent burn progression through their capacity to activate a number molecular pathways involved in tissue healing.

Human bone marrow-derived MSC were conditioned in serum-free media in 2.5% oxygen for 7 days. Conditioned media was concentrated tenfold using a 5 kD membrane. A rat comb burn model was used to generate serial full thickness burns with viable interspaces. Rats (n=12) received either two daily applications of conditioned media or vehicle. Digital photographs, Laser Doppler imaging, and punch biopsies of interspaces were obtained daily for seven days. Imaging software was used to calculate percentage of interspace conversion and perfusion on each post-burn day. Punch biopsies were embedded and sectioned to quantify epidermal thickness as well as apoptosis and necrosis.

Burn progression within viable interspaces was significantly reduced in the treated rats compared to sham rats on each post-burn day (1.25% vs 27.2% on day 1, 16.2% vs 47.3% on day 2; $p < 0.05$). Perfusion in each interspace

was significantly increased in the treated rats compared to sham rats (1054 vs 559 PU on day 3, 995 vs 576 PU on day 4; $p<0.05$). Treated burns demonstrated a trend toward increased epidermal thickness and decreased apoptosis compared to sham.

Mesenchymal stem cell-derived conditioned media is a novel topical therapy to prevent burn progression. Future study is warranted to evaluate the molecular and cellular mechanism of the therapeutic effect.

P.LB07 - USE OF AN ANTIMICROBIAL MICROFILM WOUND DRESSING IN SPONTANEOUS WOUNDS IN ANIMALS

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A composite antimicrobial microfilm dressing was utilized for treatment of spontaneous wounds on animals presented for treatment at a veterinary medical teaching hospital. The microfilms were polyvinyl alcohol hydrogel sheets with a polymeric coating containing ionic and metallic silver. They have very low amounts of silver, with 0.1 mg/sq. inch. In vitro, they reduced 5 log units of bacterial loads on their surface for >3 days. At the veterinary hospital, owners of the animals gave informed consent and treatments were initiated after conventional wound therapies were ineffective. Thirty three animals were treated using microfilm dressing, including dogs (25), cats (5), 2 foxes and 1 horse. Wounds treated included acute traumatic wounds, chronic nonresponsive wounds up to 5 months duration, postsurgical wound dehiscences with infection and necrosis, dehiscence with exposed bone plates, decubitus ulcers, infected necrotic elbow hygromas, over a skin graft and intraincisional use in one dog with a contaminated neoplasm excision site. When applied to a moist wound surface, microfilms absorbed wound fluid and conformed to wound-bed. No negative sequelae from application of microfilm dressings were observed in any animal. Microfilms were typically applied with each bandage change that involved daily changes initially followed by longer intervals as the wounds healed. Microfilms sloughed off as wounds healed and was rinsed off with saline at bandage changes. Bacterial colonization of wounds included a wide variety of organisms including methicillin-resi, pseudointermedius and schleiferi spp. The impact of treatment was most easily observed in chronic wounds where chronically nonresponsive wounds began to epithelialize and reduced in size not long after starting the treatment. This beta testing shows that microfilm dressing is safe and provides a valuable adjunctive wound management tool for treatment of both chronic and acute wounds in a disparate animal population.

P.LB08 - ULTRATHIN DISSOLVABLE ANTIMICROBIAL WOUND DRESSING IS SAFE AND EFFECTIVE IN PATIENTS WITH COMPLEX CHRONIC WOUNDS

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Chronic wounds have a burden of bacteria living deep in the wound-bed that is not cleared by, or rapidly returns after, surgical debridement. MicrolyteAg is an FDA approved ultrathin dissolvable wound dressing with a polymeric nanocoating impregnated with ionic and metallic silver. The microfilm dressing adheres to soft tissue surfaces and provides sustained intimate contact of active agents with the wound-bed. MicrolyteAg has been shown to be effective against a variety of organisms including killing 5-log10 counts of MRSA and VRE in simulated wound fluid. This study evaluates the 30 day safety and efficacy in humans.

This study was an IRB approved prospective evaluation of the use of MicrolyteAg in a variety of chronic wound patients treated in our wound center. 20 consecutive patients were consented and treated with MicrolyteAg during their previously scheduled wound clinic appointments. Wound measurements and photographs were obtained during each clinic visit.

Wound etiology included postoperative infections (2), diabetic foot ulcers (4), cellulitis (1), venous stasis ulcers (7), and non-pressure chronic ulcers (6) patients. Wounds were present for an average of 145 days (range 32 to 360 days) prior to treatment. Average follow-up was 21 days. 14/20 (70%) of wounds improved with an average of 64% (range 6% to 100%) reduction in wound size (cm2). 5/20 (25%) of wounds had an average increase in size of 0.87 cm2. One patient (5%) with a chronic venous stasis ulcer had wound size increase from 37.74 cm2 to 80 cm2. This patient developed cellulitis that required antibiotic therapy.

These results suggest that MicrolyteAg is safe and effective in the treatment of chronic wounds and is a useful adjunct to currently available therapies.

P.LB09 - A CASE REVIEW SERIES OF NEGATIVE PRESSURE WOUND THERAPY WITH INSTILLATION AND DWELL TIME (NPWTI-D) USING HYPOCHLOROUS ACID (HOCL) VERSUS SODIUM HYPOCHLORITE (NAOCL) OR 0.9% NORMAL SALINE INSTILLATION IN COMPLEX INFECTED WOUNDS

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Christiana Care has been utilizing NPWTi-d with good outcomes. Provider preferences included a variety of instillation therapies such as normal saline and NaOCl solution*. This case review series of 12 patients will analyze our results with HOCl solution^ as compared to NaOCl solution* or 0.9% normal saline as the instillation therapy.

The primary objective of this study was to analyze the effects of standardizing NPWTi-d irrigant with HOCl solution^ on patient care and clinical outcomes in complex infected wounds.

This is an observational case series of medically complex patients with multiple comorbidities and grossly infected wounds with multidrug resistant pathogens. The primary endpoints of the study are: length of hospital stay, number of procedures requiring the operating room, and days to closure of wound. Our standard NPWTi-d of 10 minutes of dwell time every 4 hours was employed. A HOCl wound irrigant^ was used for its bacteriostatic and biofilm disruptive properties. Patients with complex wounds that appeared grossly infected on clinical examination met our inclusion criteria. Cultures from operative debridements were obtained. If necrotic tissue was observed, this was debrided prior to wound vac applications. We compared the results to our previous study of NPWTi-d in which NaOCl solution* or 0.9% normal saline was used as the irrigant.

Five of the twelve patients were able to be closed either by delayed primary or secondary intention. The remaining seven patients have demonstrated remarkable improvements. Mean operating room visits decreased from 7 per patient with traditional NaOCl solution* or 0.9% normal saline to 2.8 visits utilizing the HOCl solution^. Days to closure improved from 37 to 27.4 days, respectively. Finally, mean length of stay decreased from 25 to 14 days. Diagnoses included necrotizing soft tissue infections, necrotizing fasciitis, polymicrobial abscesses resulting in compartment syndrome, stage IV infected sacral ulcer, and complex abdominal or extremity multidrug resistant abscesses.

Our experience with using HOCl irrigation therapy^ has shown promising results with a decrease in hospital length of stay, operative visits, and days to closure. These favorable outcomes will need to be followed closely as we continue to use NPWTi-d with HOCl solution^ irrigation in grossly infected complex wounds at our institution.

* Dakin's Solution® ^ Vashe Wound Irrigant®

P.LB10 - UNIQUE CONTRACTILE PHENOTYPE OF FETAL FIBROBLASTS PREVENTS MYOFIBROBLAST DIFFERENTIATION

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During post-natal or “adult” dermal wound healing, wound rigidity and transforming growth factor-b1 (TGF-b1) induce fibroblasts to differentiate into myofibroblasts. Myofibroblasts utilize stress fibers rich in a-smooth muscle actin (a-SMA) to generate large contractile forces that are transduced via focal adhesions to excessively contract and remodel the extracellular matrix (ECM) leading to scarring. In contrast, injured skin in the fetus heals scarlessly without myofibroblast involvement suggesting that fetal fibroblasts exert smaller cellular forces due to a unique response to their wound environment. However, fetal wounds are more compliant than their adult counterparts and have less TGF-b1. Therefore, it remains unclear whether the lack of myofibroblast differentiation is a result of inherent characteristics of fetal fibroblasts or biomechanical and biochemical environmental differences. In this study, we tested whether physiological rigidities and TGF-b1 can regulate fetal fibroblast contractility and induce myofibroblast differentiation. Using traction force microscopy and polyacrylamide gels (PAAs) that mimic the mechanical properties reported for the different stages of healing wounds, we found that traction force generation and focal adhesion formation by fetal fibroblasts were impaired on rigid PAAs that mimic late stage granulation tissue when compared to adult fibroblasts. When cells on rigid PAAs were stimulated with TGF-b1, we found that fetal fibroblasts showed no changes in traction forces, focal adhesions, or a-SMA while all three increased for adult fibroblasts indicating myofibroblast differentiation. Overall, our data suggest that fetal fibroblasts exhibit a unique contractile phenotype that prevents myofibroblast differentiation due to altered mechanical responses to ECM rigidity.

P.LB11 - COMPARISON OF INTRADERMAL AND SUBCUTANEOUS TISSUE OXYGEN TENSION MONITOR TO DETECT FLAP COMPROMISE

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Purpose: The ideal monitoring tool to evaluate free flap success should be minimally invasive, continuous, and reliable. Our group has previously introduced implantable oxygen sensors to monitor flaps in the immediate post-operative period and detect acute vascular-compromise. Purpose of this study was to compare intradermal vs. subcutaneous implantation of sensors in their ability to detect flap-compromise.

Methods: Experimental sensors were made by incorporating benzo-porphyrin-dye into a matrix of biocompatible hydrogel. These sensors were approximately 3mm-long, 1.5mm-wide, and 0.5mm-thick. Two groups of male Sprague-Dawley rats had skin flap-site outlined and three sensors were intradermally (ID) implanted at tip, middle and base of impending flap of one group, while subcutaneously (SQ) implanted in second group. Corresponding control-sensors were implanted laterally at least 1cm away from the proposed flap in both groups. One day later, outlined, caudally-based, full thickness flap was elevated on dorsum of rats. Gross-flap viability was assessed with computer planimetric-analysis. Inspired-oxygen was modulated between 100% and 12%. Real-time tissue oxygen tension readings were obtained from sensors on days 0, 3 and 7.

Results: Oxygen readings by sensors modulated as expected when inspired oxygen was changed, indicating that sensors are responsive and sensitive. Gross planimetric analysis of both groups showed that 16% of flap was necrotic at tip of flap as measured on d3 and was more pronounced on d7. Readings from ID and SQ sensors have demonstrated statistically significant decreases in oxygenation in all-regions of flap at all time-points compared to control sensors. Overall, SQ implanted sensors showed faster response times than ID implanted sensors.

Conclusion: Our analysis revealed that even though both methods are efficacious and accurate in determining changes of oxygenation, SQ sensors responded faster that ID sensors, however ID implantation is easier, less invasive and keep sensor localized in the specific spot where is it implanted.

P.LB12 - RAPID DETECTION OF ACUTE VASCULAR OCCLUSION USING OXYGEN MONITORING IN A RAT MYOCUTANEOUS FLAP MODEL

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Vascular compromise commonly occurs in the immediate postoperative period in association with failure of micro-vascular anastomosis. It is estimated that 6% to 25% of skin flaps require a secondary surgical re-exploration and approximately 10% of flaps fail. Currently, all monitoring methods have limitations, suffer calibration-difficulties and are expensive. In this study we introduce implantable oxygen sensors as a new method to detect acute vascular occlusion.

Methods: Experimental sensors were made by incorporating benzo-porphyrin dye into a matrix of biocompatible hydrogel. These sensors were approximately 3mm-long, 1.5mm-wide, and 0.5mm-thick. Male Sprague-Dawley rats were used throughout the study. Sensors were implanted intradermally in impending flap site. Inspired oxygen was modulated between 100% and 12% to confirm sensor sensitivity. Superficial inferior epigastric artery (SIEA) myocutaneous flaps were surgically elevated. SIEA flap was first outlined on right ventral abdomen on location of superficial inferior epigastric vessels. These vessels were dissected to create a 3×5cm island flap containing skin, fat, and panniculus-carnosus muscle. Tissue oxygen tension (TOT) readings were obtained from implanted sensors both at baseline and during vascular clamping of the feeding blood vessels.

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

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

P.LB13 - NOVEL IMPLANTABLE OXYGEN BIOSENSORS FOR DETECTION OF VASCULAR PERFUSION AND ISCHEMIA

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Tissue non-viability due to a lack of adequate perfusion and oxygenation still remains a problem in many surgical settings, including free tissue-transfer and non-healing wounds. Similarly, during endotracheal intubation, endotracheal tube compression can lead to significant tongue necrosis, requiring extensive oral and maxillofacial reconstructive surgery. Unfortunately, lack of adequate perfusion can only be assessed post-operatively, after onset of tissue-necrosis. Here we have developed a new materials-based oxygen biosensor that can be implanted intradermally, subcutaneously, and intramuscularly for deep and superficial measurements of tissue oxygen tension (TOT) in response to changes in perfusion and inspired-oxygen.

METHODS:Porphyrin-based sensors were incorporated into poly(ethylene glycol) diacrylate hydrogels. This formulation allows direct measurements of local oxygen concentrations through changes in the phosphorescence lifetime as well as imaging via fluorescence. To investigate tissue oxygen tension, sensors were implanted intradermally and subcutaneously in rats. Sensor activity was confirmed by modulating inspired oxygen levels between 12% and 100%. Sensors were similarly implanted acutely in pigs to monitor TOT under anesthesia. To mimic ischemic-events, sensors were directly injected at various depths in pig-tongue which was then subject to tourniquet-ischemia.

RESULTS:Oxygen sensors modulated as expected to changes in oxygen-levels. Similarly, in vivo TOT could be modulated from 0 to 110 mmHg by modulating inspired-oxygen between 12% and 100%. When implanted in pigs, sensors could be monitored at 5 anatomical implantation-sites simultaneously and permitted real-time monitoring of TOT during anesthesia and euthanasia. In swine-tongue, sensors were able to immediately detect application and release of tourniquet, occluding sublingual-artery.

CONCLUSION: Overall, our work has shown fast, efficient, and inexpensive method to monitor local changes in oxygen content in real-time and detect induced ischemia and reperfusion. Implementation of such devices could represent a facile method to prevent tissue necrosis during free tissue-transfer, endotracheal-intubation, as well as many other transplant and reconstructive surgical-procedures.

P.LB14 - EXPRESSION OF MARKERS FOR PERICYTES AND MYOFIBROBLASTS IN BLEOMYCIN-INDUCED DERMAL FIBROSIS: POTENTIAL ROLE OF NEUROPEPTIDE RECEPTORS IN A MOUSE MODEL FOR SCLERODERMA

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PURPOSE: Scleroderma (SSc) is a chronic collagen-vascular disease that manifests initially with dermal fibrosis, then later progresses to multiple organ fibrosis. There is no treatment to arrest SSc. Recently, a mouse model of SSc was reported, in which Bleo is injected intradermally (ID) for 21-28 days. Two labs recently showed that reactive oxygen species (ROS) is associated with dermal fibrosis. We previously demonstrated that ROS trigger GRP-mediated pulmonary fibrosis to hyperoxia or radiation. In our studies, we verify that ROS triggers dermal fibrosis, and now test the hypothesis that gastrin-releasing peptide (GRP) from cutaneous nerves has a role in this process by activating on 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 females were injected intradermally with Bleo (100-µg) 5d/wk for 3-wks. Mice also received the antioxidant N-acetylcysteine (NAC) IP, and other Bleo mice received GRP blocking mAb-2A11. After 21d, lesions were immunostained for SMA, NG2, GRPR, or NMBR. Relative extent of immunostaining in dermis and epidermis was scored by 2 observers on a scale from 0-3, comparing prevalence of (+) cells (0, 1=detected in few cells, 2=many cells (+), and 3=most cells positive).

RESULTS: Bleo induced >10-fold increase in pericytes & myofibroblasts, in dermis ($P<0.001$), and NG2 and SMA staining scores were linearly correlated ($R^2 = .87$, $P<0.05$). 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 study groups. NMBR scores were unchanged amongst the groups.

CONCLUSION: In the mouse model of scleroderma, increased pericytes and myofibroblasts occur in regions of dermal fibrosis. Although GRPR &/or NMBR could contribute to Bleo-induced dermal fibrosis their expression is unchanged between groups. We previously determined that GRP induces GRPR gene expression. Regardless, sustained epidermal expression of both receptors would be consistent with potential GRP signaling in epidermis as a mechanism.

P.LB15 - NOVEL APPLICATION OF HIGH-THROUGHPUT CHROMATIN IMMUNOPRECIPITATION SEQUENCING IN FORMALIN FIXED PARAFFIN EMBEDDED DIABETIC FOOT ULCERS

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The purpose of this study was to optimize and perform high-throughput chromatin

immunoprecipitation sequencing (ChIP-Seq) using formalin fixed paraffin embedded (FFPE) human skin, specifically diabetic foot ulcers (DFUs) which, to date, has not been described in the literature. The ChIP-Seq technique allows for mapping of histone modifications and epigenetic signatures as well as binding sites/target genes for transcription factors. Previous studies have performed ChIP-Seq in keratinocyte cell lines, which may not be representative of true physiology; or fresh skin tissue, the acquisition of which may be self-limiting in research settings. Clinically, FFPE tissue represents the gold standard for storage and preservation of pathology samples, yet using FFPE specimens as the starting material for chromatin extraction is technically challenging for numerous reasons. Specifically: 1) different tissues have varying degrees of cellularity thus affecting the yield of chromatin in downstream steps; 2) in heavily crosslinked samples the epitope of interest may not be recognized by the desired antibody and, 3) the low yield of chromatin recovered at the end of the ChIP procedure requires highly sensitive quantification procedures and library preparation kits. Using a commercially available kit, chromatin was extracted from FFPE DFUs followed by ChIP with IgG and histone H3Mek4 antibodies. Preliminary validation and sequencing was performed using qPCR of known target gene GAPDH to demonstrate effective enrichment in pull down. After read mapping, peak calling, and comparison of targets Ingenuity Pathway Analysis (IPA) was used to confirm successful enrichment of 61 previously validated H3Mek4 targets that were >50 fold enriched in our H3Mek4 ChIP. In summary, this is the first description of ChIP-Seq performed in FFPE DFUs. This technology allows for

systematic and comprehensive analysis of FFPE DFUs and will serve as a powerful tool in the pursuit of novel treatments for dermatologic disease.

P.LB16 - AMBIENT TEMPERATURE VIABLE AMNION PROCESSED VIA NOVEL LYOPRESERVATION METHOD RETAINS PROPERTIES OF FRESH TISSUE

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Human placental amnion (AM) has a long history in the field of wound treatment. Advances in tissue preservation have helped to overcome the short shelf life of fresh AM and led to the commercialization of amnion products. Viable cryopreserved amniotic membrane (VCAM), which retains all components of fresh AM, has shown positive outcomes in wound management. However, cryopreservation requires specialized ultra-low temperature storage equipment, thus limiting widespread use of VCAM. Recently, a novel lyopreservation technology has been developed that allows for ambient storage of living cells and tissues. Fresh AM was lyopreserved using this method, and its tissue structure, cell viability, and wound-healing properties following rehydration were investigated in vitro and in vivo. Histological staining demonstrated that the structure of fresh AM was retained in viable lyopreserved AM (VLAM). Cell viability was assessed by calcein staining, which demonstrated that the number of viable cells was comparable in VLAM, fresh AM, and VCAM. Inhibition of tumor necrosis factor-α (TNF-α) secretion from activated immune cells, and a hypoxia-driven increase in vascular endothelial growth factor (VEGF) demonstrated VLAM's anti-inflammatory and pro-angiogenic potential in wound microenvironment assays in vitro. Furthermore, VLAM was evaluated in two wound models in diabetic mice. Similar to fresh AM and VCAM, weekly applications of VLAM resulted in significantly faster rate of wound closure compared to the control group. Moreover, wound closure in the VLAM group correlated with a decrease in pro-inflammatory cytokines and an increase in antioxidant levels, vascularization, collagen deposition and dermal thickness in wound tissue samples. These data demonstrate that both VCAM and VLAM retain the structure, cell viability, and functional properties of fresh AM. However, VLAM is stored at ambient temperatures making VLAM more accessible for widespread use.

P.LB17 - A FIRST-IN-CLASS ANTIBIOFILM TOPICAL WOUND THERAPY TO TREAT AND PREVENT BIOFILM-RELATED INFECTIONS

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This study was undertaken to assess the ability of a first-in-class series of antibiofilm antibiotic to treat and prevent biofilm wound-related infection. In an IACUC-approved porcine model of infection, excision wounds were created with n=8 wounds per experiment. Wounds were inoculated with Acinetobacter baumannii in the planktonic or biofilm phenotype. Infection was allowed to establish for 5 days after which treatment with a unique topical antibiofilm agent (referred to as CZ-1-179) was applied once daily for 2 weeks. Wounds were monitored for an additional 2 weeks to assess for recurrence of infection. For comparison to current clinical standards of care, separate wounds were likewise treated with topical silver sulfadiazine for a 2-week period. A separate swine infected with A. baumannii was treated with colistin/imipenem administered intravenously (IV) for 2 weeks. Lastly, wounds on one swine were treated with either topical CZ-1-179 or silver sulfadiazine, and the swine also received IV colistin/imipenem to determine if agents would act antagonistically. Swine were monitored for 28 days. Bacterial

counts were the primary outcome measure and analyzed statistically using ANOVA analysis. Data indicated that wounds inoculated with well-established biofilms had ~2 log10 units more bacteria compared to those inoculated with planktonic bacteria ($p<0.05$). Wound infections on the swine treated with IV antibiotics resolved, however A. baumannii were never fully eradicated, leaving wound beds still colonized with the bacteria (~3 x 10^2 colony forming units (CFU)/g tissue). In the swine treated with both IV and topical antimicrobials, silver sulfadiazine took 2 days longer to clear bacteria in wounds compared to CZ-1-179. Taken together, these data indicated that this first-in-class series of antibiofilm antibiotic may be a promising topical agent to treat and prevent biofilm wound-related infections, with potential to be used alone or in combination with current standards of care.

P.LB18 - PLACENTAL CONNECTIVE TISSUE MATRIX FOR THE TREATMENT OF RECALCITRANT CHRONIC WOUNDS

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Introduction: Chronic wounds, including diabetic foot wounds, venous stasis wounds, vasculitis wounds, pressure wounds, and other types of non-healing wounds result is significant morbidity to patients and expense to the healthcare system. Biologics are a class of treatment products that utilizes human and animal tissues or cells to provide wound modulation and promote healing. Placental Connective Tissue Matrix (PCTM) is a new human based biologic designed for the treatment of chronic wounds.

Materials and Methods: 11 patients were treated with PCTM by direct application to the wound after debridement. One radiation wound, 1 vasculitis wound, 4 venous stasis wounds, 3 post-operative pressure wounds, 1 diabetic foot wound, and 1 chronic abdominal wound. The wounds had been chronic for 6 months to 3 years and were referred after failing conventional therapy including hyperbaric oxygen therapy (HBO), biologics, advanced dressings, skin grafting and muscle and fasciocutaneous flaps. Patients were treated with one, two or three treatments of PCTM applied topically with a non-adherent dressing.

Results: One patient with a scalp radiation wound showed no improvement. Six patients showed improvement over the 3-month study period based on wound size and improved granulation. These patients included 2 venous leg ulcer patients, 2 post-operative pressure wound patients, 1 diabetic foot ulcer patient and 1 chronic abdominal wound patient. Four patients healed completely during the study period. These patients included, 2 venous stasis patients, 1 post-operative pressure wound patient and 1 patient with a vasculitis type wound.

Conclusion: PCTM can be used to treat recalcitrant chronic wounds after other treatment modalities have failed. Further studies will be needed to define optimal treatment parameters.

P.LB19 - THE USE OF DEHYDRATED HUMAN AMNIOTIC CHORIONIC MEMBRANE (DHACM) PRODUCTS FOR TISSUE MODULATION

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Introduction: Dysfunction of the inflammatory pathway can result in a variety of disease states. These disease states can act in a general manner affecting the entire body or locally affecting specific tissues or organs. Treatment of these conditions often affects the entire immune system and can have various side effects. Biologics utilize various processed human and animal tissues to promote healing and modulate local inflammation. Placental tissue specifically has been shown to modulate inflammation in various clinical states. In this series we present patients who have been treated with dehydrated human amniotic chorionic membrane products as a tissue inflammatory modulator.

Materials and Methods: 15 patients were treated with dehydrated human amniotic chorionic membrane (DHACM) products as a tissue modulator. The disease conditions were vasculitis and keloid scar prevention after excision. Vasculitis patients were treated with single or multiple treatments and keloid patients received a single treatment at re-excision.

Result: Two patients with keloid re-excision showed improvement in keloid recurrence within 6 months follow up. Thirteen patients with vasculitis type skin lesions were treated with 11 patients completely healed at 6 months follow up. Of the non-healed patients, one patient was lost to follow up and had not healed after one treatment, a second patient treated was also skin grafted and was improved but not healed after 6 months.

Conclusion: Dehydrated Human Amniotic Chorionic Membrane (DHACM) products can be used as a tissue modulator to treat inflammatory conditions. Further follow up on existing patients, a larger series and mechanistic studies will be needed to further examine this hypothesis. In addition, further studies will be needed to define optimal treatment parameters.

P.LB20 - CELL THERAPY OF BURNS: PRESENT AND FUTURE

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Skin burn injury is marked by local inflammation and cutaneous necrosis that may extend to the deep subcutaneous structures. Today, treatment is surgical excision, epidermis autograft and flap with sometimes bad results. It has been suggested that Mesenchymal Stem Cells (MSC) therapy could be used in order to treat numerous tissue lesions. We have performed a novel therapeutic approach of local radiation-induced burns by using local autologous MSC therapy combined to surgery and epidermis autograft. For this purpose, autologous bone marrow cells were collected from the unexposed iliac crest. For GMP production, MSC were expanded in a closed system containing a serum free medium supplemented with human platelet lysate as previously described (Doucet et al., J. Cell Physiol., 2005). Quality control assays evidenced that expanded cell population retained typical MSC characteristics and did not exhibit chromosomal abnormalities. As previously demonstrated, MSC produced many cytokines and growth factors which could have a critical role in improving the healing process by counteracting the local inflammatory waves and by promoting the autologous skin engraftment. MSC are also used in combination to keratinocytes for the development of a cultured epidermis for the treatment of large thermal burns. We demonstrated the benefit of this combination on biological features of keratinocytes and on functional efficiency in a mouse model of skin lesion.

We believe that MSC act as drug cells delivering in situ in the lesion growth factors which contribute to the healing of the lesion. We have also demonstrated that after in vitro cell activation, the conditioned medium of MSC exhibited a similar effect on wound healing than that obtained with freshly expanded MSC. In case of karyotypic abnormalities occurring after in vitro MSC expansion, the use of MSC conditioned medium could be considered as a relevant alternative of MSC therapy.