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THYMOSIN β_4 PROMOTES MATRIX METALLOPROTEINASE EXPRESSION DURING WOUND REPAIR

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Impaired wound healing is a problem for immobilized patients, diabetics, and the elderly. The 43 amino acid angiogenic peptide thymosin β_4 has previously been found to promote accelerated dermal wound repair in rats, aged mice and db/db diabetic mice, and corneal repair in normal rats. It has been found in great abundance in wound fluid. Here, we hypothesized that thymosin β_4 may regulate matrix metalloproteinase (MMP) expression in cells that are involved in wound repair. Western blot analysis of keratinocytes, endothelial cells, and fibroblasts that were treated with increasing concentrations of thymosin β_4 showed changes in the expression of the MMP-1, −2, and −9. Zymographic analysis of whole excised mouse wounds taken after homogenization also showed changes in MMP-2 and-9 expression over a 3-day period. These results were confirmed in 2-day-old wounds by RT-PCR. We conclude that part of the wound healing activity of thymosin β_4 resides in its ability to increase protease activity. Since thymosin β_4-induced protease activity can be further controlled by inflammatory cytokines, a regulatory role for thymosin β_4 is proposed in wound healing. These studies suggest that thymosin β_4 may play a pivotal role in extracellular matrix remodeling during wound repair and may be effective in the treatment of chronic wounds in humans.

HEALTH SERVICES POSTERS

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RETURNING SKIN AND WOUND CARE TO THE BEDSIDE: ONE HOSPITAL'S JOURNEY THROUGH PROCESS IMPROVEMENT

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An initiative to bring wound care "back to the bedside" was introduced in a large teaching hospital. Wound consultation was available from either a nurse or physician member of a hospital-based wound team. Confusion over whom to consult coupled with multiple product options led to decreased critical thinking at the bedside. The volume of consultations precluded follow-up visits by the team, further fragmenting care.
The proposed solution included partnering with a sole vendor to standardize wound products and assist with education on product selection and function. New policies and procedures were created. Each patient unit was staffed by a Nurse clinical specialist as part of a hospital-wide "Magnet" application process. This individual assumed the role of "skin care leader" and was required to attend two in-depth educational programs. All skin care leaders were required to become part of a hospital-wide quarterly prevalence and incidence project. The wound nurse assumed a more "consultative" role for the skin care leaders who subsequently assisted the end user in this model; the bedside nurse.
This paradigm shift in care did not result in any statistically significant changes in wound incidence and reintroduced skin care as an integral component of the assessment and care for the bedside nurse. This presentation will describe the process in detail.

Acknowledgments: This study was supported with an educational grant from Smith and Nephew, Largo, FL.

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CYTOKINE DYNAMICS IN THREE-DIMENSIONAL EXTRACELLULAR MATRICES

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Fluorescence correlation spectroscopy was used to measure the binding and diffusion of growth factors in model extracellular matrices in order to investigate the importance of protein-matrix interactions in regulating signaling molecules within a three-dimensional matrix. Two important growth factors were studied, transforming growth factor β1 and basic fibroblast growth factor, which are known to have specific affinities for fibronectin and the heparan-sulfate-proteoglycan perlecan, respectively. Collagen-based matrices were prepared by polymerizing type I collagen in the presence of fibronectin or perlecan, and we measured diffusion constants and binding constants of the two growth factors. The binding constant measured for transforming growth factor β1 with fibronectin-containing matrices was in good agreement with that measured using affinity chromatography. However, the interactions measured between fibroblast growth factor and perlecan were significantly weaker than expected. Surprisingly, the strongest interactions by far were with monomeric collagen solutions and fibrillar collagen matrices. Our findings suggest a central role for the three-dimensional fibrillar collagen matrix in growth factor interactions, with modulatory roles for fibronectin or perlecan.

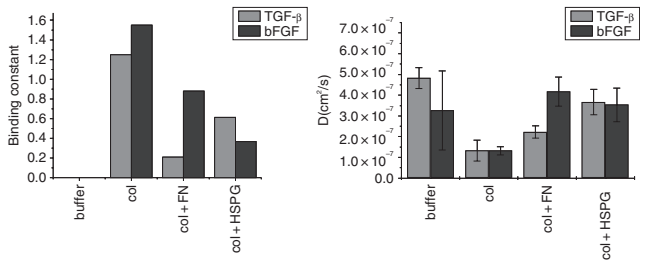

Figure 1: Binding and diffusion constants of TGF-β1 and FGF-2 measured by fluorescence correlation spectroscopy.

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TRANSITIONING CARE FROM CURATIVE TO PALLIATIVE DOES NOT MEAN ABANDONING ADVANCED WOUND CARE: EXPERIENCES FROM AN ADVANCED SUBACUTE WOUND CARE UNIT

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It is expected that by 2030, 20% of the American population will be over 65 years of age. Forty percent of Americans are expected to die in nursing homes in the near future. Pressure ulcer prevalence rates of 13% are reported at long-term care units but healing rates are infrequently published from these settings. Current wound protocols fail to consider patients goals of care. The possibility that a wound will not heal is rarely communicated to the patient or family. Palliative care focuses on the relief of suffering and improvement in the quality of life. It can be the sole focus of care or can be delivered parallel to aggressive curative treatments.
This presentation describes a case history of an unfortunate 40-year-old patient suffering from multiple sclerosis who developed a stage 4 pressure ulcer during a pregnancy. Postpartum, the patient underwent flap coverage of the wound. Her MS worsened, the flap broke down and a family conference resulted in the decision to provide palliative wound care and involve hospice. Wound care continued with a silver impregnated absorptive dressings and ultraviolet light therapy to control bioburden, diminish odor and dressing frequency, which improved quality of life. Palliative wound protocols will be reviewed along with details of the above-mentioned case.

Acknowledgments: This presentation was supported with an educational grant from Smith and Nephew, Largo, FL.

IMMUNITY POSTER

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NEGATIVE PRESSURE WOUND THERAPY (NPWT)/VACUUM-ASSISTED CLOSURE® (V.A.C.®) AS AN ADJUNCT IN THE TREATMENT OF PYODERMA GANGRENOSUM

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Aim: To determine the viability/effects of incorporating Negative Pressure Wound Therapy (NPWT)/V.A.C.® Therapy as an adjunct in the treatment of Pyoderma Gangrenosum.
Material and Methods: A 74-year-old white female presented with clinical evidence of Pyoderma Gangrenosum (PG). The wound exhibited pathergy and therefore aggressive debridement remained contraindicated. The patient applied topical Elidel cream to the periwound. With meticulous wound management, the lesion remained free of infection. Clinicians utilized various systemic treatment regimes including Minocycline 100 mg twice daily, Dapsone 50 mg daily, Cyclosporine 150 mg twice daily, and ultimately Infliximab 5 mg/kg every 2 months. The latter drug created a significant positive response; however, the wound continued to "wax and wane." Researchers began NPWT/V.A.C.® Therapy to facilitate granulation tissue, epithelization, and ultimate wound closure.
Results: The researchers observed dramatic improvement in wound dimensions, wound contracture, and granulation tissue with the addition of NPWT/V.A.C.® therapy.
Conclusions: NPWT/V.A.C.® Therapy appears to augment healing in a patient with Pyoderma Gangrenosum and may be useful as adjunctive therapy.

Acknowledgments: KCI®, San Antonio, TX

OXIDATIVE STRESS POSTER

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INHIBITION OF REACTIVE OXYGEN SPECIES IMPAIRS HEALING IN A MODEL OF TISSUE ISCHEMIA

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Reactive oxygen species (ROS) play a major role in recruiting and activating inflammatory cells, inducing angiogenesis, fibroblast proliferation, and collagen synthesis, all processes essential to wound healing. However, the same mediators have been implicated in pulmonary and hepatic fibrosis, vascular occlusion, and necrotic tissue damage. We have previously shown that hyperbaric oxygen caused a prolonged elevation of VEGF and delayed wound healing in a rat model of tissue ischemia. In this study we examine the effect of the ROS scavenger, n-acetylcysteine (NAC), on ischemic wound healing. NAC is a virtually nontoxic free radical scavenger that has been used in many experimental studies to block ROS-mediated signaling.
Methods: Male Sprague-Dawley rats underwent creation of the ischemic flap previously validated by this laboratory. This model provides two ischemic and two nonischemic excisional wounds. Rats were treated daily with HBO for 90 minutes at 2.4 atm, HBO plus 150 mg/kg NAC intraperitoneal, or control (neither HBO nor NAC). Wounds were analyzed for surface area, lactate, and VEGF.
Results: The HBO/NAC-treated animals had markedly impaired healing of their ischemic wounds at day 7 ($0.105 \pm .005$ cm vs. $0.068 \pm .002$ cm for ctl and $0.064 \pm .006$ for HBO) and a twofold elevation of VEGF (120 pg/mg protein vs. 60 pg/mg protein). Lactate levels were not altered by NAC treatment and nonischemic wounds showed no difference in healing, lactate, or VEGF.
Conclusion: Reactive oxygen species are required for wound healing. Our initial hypothesis was that the delay in wound closure with HBO treatment was due to excess ROS and that NAC would improve healing. The finding of elevated VEGF and delayed wound healing in the presence of a potent free radical scavenger suggests that blocking ROS signaling prevents the normal mitogenic response to VEGF. Additional studies to examine the VEGF receptor and tyrosine kinase activation will further elucidate the mechanism of ROS signaling in response to HBO treatment.

OXYGEN POSTERS

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TRANSDERMAL SUSTAINED OXYGEN THERAPY PROMOTES HEALING OF A CHRONIC PRESSURE ULCER IN A PATIENT WITH OSTEOMYELITIS: A CASE REPORT

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[3] SUMMA Health System, Akron, OH

Clinical Problem: An 89-year-old white male presented to his dermatologist with a pressure ulcer on his left medial foot. He was not a surgical candidate (CHF, pacemaker, edema) and not diabetic.
Past Management: For 2 years the dermatologist provided standard of care therapies treating the wound with regular debridements and Regranex therapy. The wound remained unhealed. At the age of 91 the patient developed osteomyelitis. Antibiotic therapies were initiated.
Current Clinical Approach: In addition to antibiotic therapy the patient agreed to have a continuous supply of oxygen delivered transdermally to the wound at a rate of 3 ml/hour from a device that derived pure oxygen from atmospheric air (EpiFLO^{SD}) as the patient ambulated. 24/7 oxygen therapy was used in conjunction with a fully occlusive Johnson & Johnson $3'' \times 4''$ island dressing.
Patient Outcomes: After 3 weeks of continuous oxygen and antibiotic therapies the patient was able to ambulate without pain. The planned amputation was canceled. The wound bed was characterized by new granulation tissue and reepithelialization. The antibiotic treatment was discontinued after 30 days, but the tiny, 2 ounce oxygen therapy device was continued until full wound closure 11 weeks later. The wound remained closed for 36 months, the remainder of the patient's life.
Conclusions: The effectiveness of the antibiotics in this patient as well as other oxygen-sensitive wound healing processes may have been potentiated and enhanced by the continuous availability of oxygen at this dosage. Further carefully controlled studies of transdermal sustained oxygen delivery for the treatment of chronic wounds with osteomyelitis are needed to clarify the underlying scientific mechanisms of this beneficial effect. The support of Ogenix Corporation in supplying EpiFLO^{SD} for this project is appreciated.

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HYPERBARIC OXYGEN ENHANCES WOUND LACTATE PRODUCTION IN RATS

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Introduction: Healing wounds are characterized by high lactate levels. In the past, the shift to an anaerobic energy metabolism was thought to be the main source for increased lactate concentration. Lactate is also generated by leukocytic NADPH-linked oxygenase using oxygen and glucose as one of its substrates, a mechanism called aerobic glycolysis. We hypothesize that hyperbaric oxygen therapy by increasing oxygen availability enhances wound lactate production by aerobic glycolysis.
Material and methods: Four wire mesh wound cylinders ($n = 142$) were implanted underneath the dorsal skin in each of 36 male Sprague-Dawley rats (312 ± 11 g). Animals were randomized to two groups: The first group ($n = 18$) received 100% oxygen with 2.1 ATA for 90 minutes twice a day for a total of 7 days. The second group ($n = 18$) was treated with 21% oxygen with 1 ATA for the same time schedule. Hyperbaric treatments were administered in a hyperbaric chamber designed for small animals. Wound fluid from the cylinders was aspirated between the two treatment cycles at day 2 and 5 and analysed for lactate using a lactate analyser. Additionally, at day 10 (after 2 days of treatment with 21% oxygen in 1 ATA in both groups) lactate concentrations in wound fluid were measured again. Data are expressed as mean $\pm$ SD. A p-value <0.05 was considered significant.
Results: Wound lactate concentration was significantly increased both in control and hyperbaric rats at day 5 and day 10 compared to day 2 (controls 4.5 ± 1, 5.9 ± 0.8, 6.7 ± 1.2 mmol/l; $p = 0.04$ and hyperbarics 4.4 ± 1.2, 6.5 ± 1.3, 8.2 ± 1.3 mmol/l; $p = 0.01$). At day 2 and day 5 there was no significant difference in lactate levels between control and hyperbaric rats. However, at day 10 lactate concentration was significantly higher in the hyperbaric group compared to the control group (6.7 ± 1.2 vs. 8.2 ± 1.3 mmol/l; $p = 0.01$).
Conclusion: This investigation provides evidence for the mechanism of hyperbaric oxygen action since lactate is known to stimulate collagen production and angiogenesis.

ACUTE WOUNDS POSTERS

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SURFACTANT CHAPERONE P188 SEALS ELECTROPORATED MUSCLE MEMBRANES

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Introduction: Electric fields of the magnitude likely to occur in electrical injury can result in skeletal muscle electroporation. It has been reported that Poloxamer 188 (P188) is able to seal damaged cell membranes. Using a previously described rat hind limb model of electrical injury, in this study we employ MRI to demonstrate that intravenously administered P188 arrests muscle tissue edema caused by electrical shock.
Methods: Anesthetized Sprague-Dawley rat hindlimbs were subjected to 12 electrical shock pulses of 2 kV and ~2A amplitude with duration of 4 ms. There was a 10-second separation period between shocks to allow thermal relaxation. Animals received either P188 or Lactated Ringer's Injection (control) intravenously at 60 minutes after shock. Proton MRI were recorded using a 4.7-Tesla Bruker scanner (200 MHz). A Multi-Slice Multi-Echo (MSME) spin echo sequence was used to obtain T_2-weighted images. Ten slices over the entire midthigh region were taken. For each slice, images from 10 echos in the TE range of 10 ms–100 ms and TR = 2000 ms were collected. Imaging began at 60 minutes and continued until 3 hours postshock. The signal intensities at different TE times were used to fit an exponential decay and measure the T_2 value in each voxel. Voxels with T_2 values > 80 ms were considered injured.
Results: There were noticeable increases of injured area with time for electrically injured limbs due to development of edema. Control-treated rats averaged a 58.0% (±6.59% SD) increase in total area of injury. The total area of injury for P188-treated rats increased by 46.6% (±2.96% SD) In nonelectroporation limbs, no change in T_2 occurred.
Discussion: Disruption of the plasma membrane due to electrical current leads to edema. We treated the injured muscles with P188 and observed that it helped to arrest edema. MRI proved effective not only in characterizing the degree of injury resulting from electrical shock but also in monitoring therapeutic efficacy.

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LOCAL INJECTION OF INSULIN-ZINC SUSPENSION STIMULATES DNA SYNTHESIS IN SKIN DONOR WOUND

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We have previously reported that local injection of small doses of long-acting insulin-zinc suspension maintained sufficiently high insulin concentration in the wound fluid and accelerated the healing. The present experiment was to measure the rates of cell proliferation (reflected by DNA synthesis) and protein metabolism in the wound. These are the underlying metabolic processes responsible for the healing. Partial thickness skin donor wound was created on the back and indwelling catheters were placed in the carotid artery and jugular vein in adult rabbits under general anesthesia. The wound was covered with Aquaphor gauze, OpSite membrane, and surgical gauze. On day 7 after wounding, the wound was either injected with 0.2 units of insulin-Zn, Zn alone, or no injection; stable isotopes were infused into the jugular vein catheter in conscious rabbits for measurement of DNA synthesis, protein synthesis and breakdown in the wound. The local insulin-Zn injection raised wound insulin concentration to 168 ± 39 μU/ml. In contrast, in the control and Zn groups wound insulin concentrations were below the detectable level. The local injection of insulin-Zn suspension increased ($p = 0.03$) DNA synthesis in the wound with minor changes in blood glucose concentration which did not require exogenous glucose replacement. In the Zn group, whereas the rate of DNA synthesis tended to increase ($p = 0.051$ vs. control), the protein net balance (synthesis – breakdown) was lower ($p < 0.05$) than those in the control and insulin-Zn (Table 1). We conclude that local injection of a small dose (0.2 units insulin) long-acting insulin-Zn suspension stimulated wound DNA synthesis without major systemic side effects thereby providing an effective and safe approach to accelerate wound healing. Although local Zn injection might also stimulate wound DNA synthesis, the benefit on the healing process was limited by decreased net protein deposition.

Table 1. Wound DNA and protein metabolism.

	DNA synthesis	Protein synthesis	Protein net balance
Control (n = 7)	2.9 ± 0.9	20.5 ± 8.4	7.9 ± 6.0
Insulin-Zn (n = 7)	4.2 ± 0.9*	17.9 ± 8.2	9.4 ± 6.4
Zn (n = 7)	4.1 ± 0.9+	13.0 ± 5.8	−0.2 ± 3.4#

Values are means ± SD in %/d. * $p < 0.05$ vs. control; + $p = 0.051$ vs. control; #, $p < 0.05$ vs. control and insulin-Zn by one-way ANOVA.

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DERMAL WOUND HEALING IN AN ANIMAL MODEL FOLLOWING THERMAGE TREATMENT

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The Thermage system is a radiofrequency (RF) device designed to treat human skin. The Thermage ThermaCool TC™ is a noninvasive, nonablative, nonlaser system that combines cryogen cooling and radiofrequency (RF) energy to volumetrically heat dermal and subdermal tissue. Thermage treatment causes collagen contraction resulting in immediate skin tightening. It has also been clinically shown to tighten skin over time. This long-term tightening effect is believed to result from the body's wound healing response. We performed studies in a juvenile pig to characterize the wound healing response after ThermaCool treatment in order to elucidate the cellular and molecular events resulting from treatment. Histological markers of the wound healing process were examined over a 1-month period. Collagen gene expression was examined by quantitative PCR.
Macrophages and mast cells are important inflammatory cell types that stimulate fibroblast recruitment, collagen synthesis and blood vessel growth. Three to 5 days following treatment, macrophages and mast cells were present in the dermis. By 28 days, both increased dermal blood vessel density and epidermal thickness was observed. Collagen I and collagen III genes were expressed at elevated levels by 21 days posttreatment.
ThermaCool™ treatment stimulates dermal remodeling by initiating a wound-healing response. This study demonstrates that thermage treatment results in the recruitment of important cellular mediators of wound healing and increased collagen synthesis in an animal model. These events are presumed to be responsible for dermal remodeling and skin tightening over time in human subjects. Because the Thermage device noninvasively delivers heat energy, it may be a unique tool for the examination of aseptic thermally induced wound models.

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THE USE OF WET-TO-DRY WOUND DRESSINGS

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Purpose: The purpose of this study was to determine the current use of wet-to-dry dressings as the principal wound care modality ordered by health care providers. In addition, this study describes the types of wounds wet-to-dry dressings are currently used for, the specialty of health care providers most likely to order these dressings, and associated data that indicates whether or not the clinical condition of the wound warrants the use of wet-to-dry dressing for mechanical debridement.
Method: This descriptive study involved a retrospective chart review of 202 home health and HMO case managed patients with open wounds One hundred and two HMO charts were reviewed; only 74 had sufficient data documented. One hundred and sixty-one home health charts were reviewed; only 128 were appropriate for data collection (closed incisions, drainage tubes, ostomies, and those charts where more than six items on the data collection sheet were not documented in the chart, were not used). The data was analyzed using SPSS.
Findings: This study suggests that the current use of wet-to-dry dressings is over 41% of all principal wound care modalities, followed by enzymatic (7.43%) and dry gauze (6.93%). The majority of wet-to-dry dressings ordered are for surgical wounds (69%), followed by neuropathic ulcers (10%) and pressure ulcers (5.9%). Furthermore, this study suggests that general surgeons are most likely to order wet-to-dry dressings (38%). Data also indicated that in over 78.6% of wounds treated with wet-to-dry dressings, mechanical debridement was not indicated (the percent of granulating tissue in the wound bed was > 75%). In only 3.5% of wounds treated with wet-to-dry dressings was mechanical debridement possibly indicated by the amount of nonviable tissue in the wound bed.
Discussion: Clinicians need to be involved in research that will lead to evidenced based clinical practice and standardized wound care. The scientific literature suggests that wet-to-dry dressings are not evidenced based, but are detrimental to the granulating wound bed, painful, costly, and one of the least effective methods of debridement available today.

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NEAR INFRARED SPECTROSCOPY OF PARTIAL THICKNESS BURNS

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Introduction: This study examines the capacity of near infrared spectroscopy (NIR) to differentiate partial thickness burns.
Methods: Adult burn patients ($n = 19$) presenting within 72 hours of injury and body surface area < 20% were studied. An independent observer classified the burn injuries as either superficial (SPT, $n = 5$) or deep partial (DPT, $n = 14$) thickness. NIR data, oxygen saturation and total hemoglobin, were collected from the burn site and adjacent nonburned control site. NIR data for burn sites were compared to respective control sites to adjust for between subject variability. Skin biopsies for histologic analysis were performed at the time of surgery.
Results: NIR spectroscopy was able to detect a slight increase (3.65%, $p < 0.05$) in oxygen saturation with SPT burns when compared to control sites. In contrast, DPT burns displayed a drop in oxygen saturation (17.65%, $p < 0.05$) in comparison to the control site. An increase in total hemoglobin was observed for both the SPT (15.83%, $p < 0.05$) and DPT (5.07%, $p < 0.05$) injuries in comparison to control sites. SPT and DPT burns are discernible ($p < 0.01$) based on oxygen saturation values but not total hemoglobin ($p > 0.4$). Histologic and clinical correlation with the NIR spectroscopic data will be presented at the conference.
Conclusions: NIR spectroscopy can distinguish between superficial and deep partial thickness burn injuries in the first 3 days post burn injury.

PSI grant

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HYALURONAN AND ACUTE DERMAL WOUNDS

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Introduction: Hyaluronan (HA), a glycosaminoglycan found in many tissues, is most highly concentrated in the dermis and epidermis of the skin. HA is believed to have an important role in wound healing. HA is thought to be actively produced during wound healing and tissue repair to provide a framework for ingrowth of blood vessels and fibroblasts. The present study was performed to investigate the histology, levels, and molecular weight of hyaluronan in human open dermal wounds.
Methods: Dermal wounds were created using a 4 mm punch biopsy on the buttocks of 10 healthy adult subjects. Wounds were harvested using a 6 mm punch biopsy at postwound days 1, 3, 7, 14, and 28. Tissue was then extracted for determining hyaluronan levels using a biotin hyaluronic acid link protein assay. The molecular weight distribution of hyaluronan was examined using agarose gel electrophoresis. Histology was performed using HA biotinylated binding protein with hyaluronidase controls.
Results: The levels of hyaluronan were found to have a bimodal distribution within the 28-day time course. Levels of HA increased from baseline values 12.4 ± 6.3 to 47.3 ± 7.5 ($p < 0.05$) on postwound day 1.
The lowest HA levels were found on postwound day 3 (26.4 ± 8.5), with levels again increasing on postwound day 7 to the highest levels 73.4 ± 13.6 ($p < 0.05$). Postwound days 14 and 28 showed levels gradually declining toward baseline (49.4 ± 5.7, 43.9 ± 6.8). Molecular weight analysis showed smaller size HA on postwound day 1. Histological analysis demonstrated the presence of hyaluronan in the granulation tissue of healing wounds.
Conclusion: This is the first detailed examination of HA expression in human wounds. The bimodal display of HA levels in these wounds suggest that HA may be subject to fragmentation at times when the inflammatory index of these wounds is greatest.

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FACTS AND THEORIES OF ORGAN REGENERATION IN ADULTS

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Induced organ regeneration is de novo synthesis of a physiological, or nearly physiological, organ at the same anatomical site as the organ that is being replaced. Regeneration of skin, peripheral nerves, and the conjunctiva have been accomplished using nothing more than biologically active scaffolds (regeneration templates) seeded with epithelial cells; devices for regeneration of the first two organs are in clinical use. Templates appear to function by blocking contraction well as by acting as temporary configurational guides for synthesis of new stroma that resembles that of the organ under replacement. The combined evidence supports a theory which predicts that selective blocking of the adult healing response uncovers part, at least, of the latent fetal response to injury and, in the presence of the appropriate scaffold, eventually leads to organ regeneration. An independent theory suggests that loss of regenerative potential, which is observed during the mammalian fetal-adult transition, is associated with simultaneous acquisition of individual immunocompetence.

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BISMUTH SUBGALLATE/BORNEAL (SUILE) VS. BACITRACIN IN THE HUMAN FOREARM BIOPSY MODEL FOR ACUTE WOUND HEALING

T. Serena[1, 2, 3] and V.W. Li[2] on behalf of the Wound Healing Cooperative Group; L.K.S. Parnell[4], M. Miller[5], K. Shay[3], M. Brown[3], M. Wilt[3]
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Background: The human forearm biopsy model is employed by the Wound Healing Cooperative Group to evaluate the effect of novel agents on acute wounds. Bismuth Subgallate/Borneal (Suile) is a new product with FDA permission for the treatment of partial thickness wounds. However, there is a growing body of evidence suggesting that Suile may be effective in the treatment of full thickness wounds because of its antimicrobial, hemostatic and antiinflammatory properties.
Methods: In our randomized, investigator-blinded study, 20 normal healthy volunteers underwent two 6 mm full-thickness skin biopsies on the flexor surface of each forearm (two wounds per subject). Biopsy sites were randomly assigned to control (daily bacitracin) or to the treatment arm (daily Suile). The wounds were examined, measured by digital planimetry, and photographed daily until complete healing was achieved. Adverse events and pain levels were monitored. Healing velocity and time-to-complete closure were determined.
Results: The forearm biopsy model allowed direct quantitative and qualitative comparisons of wound healing outcomes achieved by the Suile regimen versus bacitracin alone. Subject compliance was excellent. The conduct of the study was further facilitated by the use of handheld electronic documentation.
Conclusion: The forearm biopsy model has several advantages: It allows for a direct comparison of topical agents in a controlled fashion, studies can be conducted rapidly with good patient compliance and it allows investigators to gain firsthand experience with products prior to embarking on large clinical trials in chronic wounds.

Acknowledgments: Grant from Hedonist Biochemical Technologies Co.

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BASIC FIBROBLAST GROWTH FACTOR SUCCESSFULLY IMPROVES WOUND HEALING WITH ARTIFICIAL SKIN SUBSTITUTE

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2 Division of Anatomy and Neurobiology
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Acute wounds caused by the severe and extensive infection such as alpha-hemolytic streptococci or MRSA may be life-threatening in immunocompromised hosts such as steroid users or those with venostasis in the lower legs. The principle of the treatment is the removal of the primary pathogens, however, difficulties of the lower leg reconstruction remain due to the tissue loss and the deficiency of the circulation system after extensive soft tissue debridement. For 10 immediately-developing extensive lower extremity cases, artificial skin substitutes composed of dried bilayer membranes of outer silicone membrane and inner porcine-tendon derived collagen layer (Pelnac®, Gunze Co., Ltd., Kyoto, Japan) were immediately after extensive and meticulous debridement. While the skin substitute integrated into the wound bed for maximally 3 weeks, total 20 micrograms of the basic fibroblast growth factors (bFGF) (Trafermin®, Kaken Pharmaceutical Co. Ltd, Tokyo, Japan) were applied by the 27-Gage syringe daily. The outer membranes were removed and thin partial thickness skin grafting (9–10/1,000 inches) was performed and wound healed uneventfully.
The bFGF-treated skin texture was significantly softer compared to control by a durometer (Teclock®, GS-701N, Tokyo, Japan)($p < 0.01$), which was a measuring device elastic hard products such as rubbers and polymers.
Reconstruction using the porcine-derived artificial skin substitute in the lower extremities after extensive debridement was safe, easy, and reproducible. The quality of the wounds with bFGF significantly improves the healed skin texture.

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CORRELATION OF ACUTE HUMAN AND MURINE WOUND HEALING WITH AND WITHOUT TOPICAL GROWTH FACTOR THERAPY

W.W. Li and V.W. Li on behalf of the Wound Healing Cooperative Group (WHCG) and
The Angiogenesis Foundation, Cambridge, MA

Background: Previously, we showed in a human forearm biopsy model of wound healing that acute healing dynamics can be characterized at the clinical and genetic level. Growth factor application by recombinant human platelet-derived growth factor-BB (rhPDGF-BB) accelerates the velocity of wound healing and modulates gene expression in wounds. To profile the function, structure, and genomics of acute healing, the human results were compared with a murine model of acute circular and incisional wounds.
Methods: Full thickness 8 mm circular skin wounds and linear surgical incisional wounds were created in 6-week-old Balb-C mice and recombinant angiogenic growth factors (PDGF vs. PDGF/FGF2/VEGF) were administered topically to wounds daily. Velocity of healing and time to complete closure were determined by digital planimetry. Tissue was examined for histopathological, morphometric, functional, and gene expression parameters, and compared to human results.
Results: Acute healing velocity can be quantified and compared in human and murine models. Linear incisions heal in distinct morphological, immunohistochemical and gene expression patterns compared to circular wounds. The cellular profile shows differences between wound types. Topical PDGF accelerates angiogenesis and time to complete acute healing. Human and murine gene expression was correlated. Results will be presented.

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HIGH-RESOLUTION MRI RELAXATION OF WATER PROTONS IN PROTEINS AND ELECTROPORATED RAT MUSCLES

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Introduction: Electroporation of cells leads to breakdown of the ion gradients and release of intracellular contents. This triggers edema, which raises the local hydration level over its physiological value. Therefore, the state of water in an injured tissue is changed and the compartmentalization and dynamics of the spin populations during magnetic relaxation differ from healthy tissue.
Methods: We measured spin-spin relaxation time (T_2) and spin density (M_0) parameters of water protons in electroporated rat muscles by using a 4.7-Tesla GE/Bruker Omega scanner. The rats were sacrificed 3 h postshock and *extensor digitorum longus*, *soleus* and *biceps femoris* muscles were collected. Contralateral muscle samples served as controls. T_2 and M_0 were obtained using Carr-Purcell-Meiboom-Gill methods. Following MR imaging the water content in the tissue samples was measured. To infer T_2 from the value corresponding to bulk water we also measured T_2 of *lysozyme* and *ribonuclease* (1 mM) solutions (10 mM phosphate buffer at pH = 7.0) as a function of protein concentration.
Results: T_2 values were determined by fitting the signal amplitude from the echoes to the decay time. We obtained for deionized distilled water $T_2 = 614 \pm 31$ ms, for lysozyme, $T_2 = 478 \pm 25$ ms and for ribonuclease A, $T_2 = 423 \pm 22$ ms. The analysis of T_2-weighted MR images in rat muscles showed a 25–75% increase of the water T_2 relaxation time in the three different muscle samples, of which *soleus* muscle showed the largest increase of 75%, from ~40 ms in controls to ~70 ms in injured muscles.
Conclusions: Electroporation leads to increased in T_2, which may have two components. First, the accumulated water adds extra hydration layers to proteins. Second, the dynamics of water molecules may be altered as a result of development of the structural damage due to activation of degradative processes in the tissue.

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APPLICATON OF A NOVEL CROSSLINKED GLYCOSAMINOGLYCAN HYDROGEL SCAFFOLD IMPROVES HEALING IN A RABBIT EAR ULCER MODEL

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Introduction: Hyaluronic acid (HA) is a major glycosaminoglycan component of normal dermis and a major component of the matrix synthesized during wound healing. Previous studies have shown that uncrosslinked HA is rapidly degraded and cleared in vivo, limiting its clinical utility. We have used a novel HA hydrogel scaffold that is crosslinked for prolonged bioavailability. It has a porous interior and a smooth surface. This scaffold can also be fabricated with collagen and linked to proteins or DNA to direct cell growth and differentiation. We hypothesized that this novel HA formulation might itself improve wound healing by enhancing cell migration.
Purpose: The present study explored the effects of a crosslinked HA hydrogel on wound healing.
Methods: Using an established model of wound healing, four 7 mm punch wounds were made on each ear of New Zealand rabbits. Each wound was immediately treated with topical application of one of the following: HA hydrogel ($n = 26$), HA/collagen hydrogel ($n = 27$), or phosphate-buffered saline (PBS) ($n = 16$). Wounds were harvested on day 8 for histologic evaluation. Sections were examined for epithelial and granulation tissue ingrowth into wounds.
Results: HA- and HA/collagen-treated wounds showed statistically significant increases in epithelial ($p = 0.003$ and $p = 0.007$, respectively) and granulation tissue ingrowth ($p = 0.004$ and $p = 0.01$, respectively) when compared to PBS-treated controls.
Conclusions: In the rabbit ear ulcer model, the application of crosslinked HA and HA/collagen hydrogels accelerated wound healing as measured by epithelial and granulation tissue ingrowth.

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CAN PLATELET-RICH PLASMA GELS ENHANCE EPITHELIZATION OF DEEP PARTIAL THICKNESS WOUNDS?

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Background: Platelets are a rich source of proteins and growth factors that are instrumental in the initiation and modulation of tissue repair. The clinical use of crude platelet preparations has been around for decades. However, the use of autologous platelet gel (APG) for the treatment of wounds has become familiar practice in only the past 10 years. Platelet gels have been associated with accelerated vascular ingrowth, increased fibroblastic proliferation, and accelerated collagen production.x3
Objective: The purpose of this study was to examine the effects of APG using a porcine wound healing model.
Methods: Platelet rich plasma (PRP) was obtained using a fully automated, easy-to-use devise. APG was created from PRP and autologous thrombin. Deep partial thickness wounds were created on specific pathogen-free pigs. Wounds were either treated with APG and covered with a polyurethane dressing, were treated only with a polyurethane dressing, or were left untreated. Wounds were assessed for epithelization using a well-established salt-split technique.
Results: Wounds treated with APG enhanced the rate of epithelization as compared to both polyurethane and untreated controls.
Conclusions: This study shows that an autologous platelet rich gel can stimulate and enhance epithelization of deep partial thickness wounds. Additional preclinical and clinical studies that examine mechanisms of action are warranted.

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DIFFERENTIAL REGULATION OF ANGIOGENIC GENES IN NORMAL AND DIABETIC WOUND HEALING

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Wound healing is a complicated biological process that involves interactions of multiple cell types, various growth factors, their mediators and the extracellular matrix proteins. In this study, we have studied the differential regulation of angiogenic genes during wound healing in transgenic diabetic mice and nondiabetic mice. One 8 mm full thickness cutaneous wound under aseptic conditions was created on either side of the midline. Wound tissues were studied at 4, 7, and 11 days postwounding and healing was assessed by histology. The pathway specific gene expression profile of wound tissue in transgenic diabetic mice was compared with the normal mice. Profiling of these genes showed differential regulation of many angiogenic promoters, inhibitors, growth factors and cytokines. Furthermore, in our study hypoxia inducible factor (HIF-1a), osteopontin (OPN) and osteonectin are induced early at day 4, in both the diabetic and nondiabetic wound. The expression was downregulated by 11-day postwounding in the nondiabetic wound, whereas diabetic wounds showed constitutively high expression of these genes. The expression patterns of these genes were concomitant with the extent of healing as assessed by histology at different time point postwounding. These results suggest wound healing is a complex process that involves cascade of interaction of various factors. Although a single gene may not be solely responsible for any impairment in healing; however, an in-depth study of these genes and precise balance between the inducers and inhibitors of angiogenesis may provide an answer to the delayed healing in diabetic conditions.

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A RANDOMIZED CONTROLLED STUDY COMPARING VACUUM ASSISTED CLOSURE TO STANDARD DRESSING CHANGES IN THE DEVELOPMENT OF ANGIOGENESIS

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Hypothesis: The use of Vacuum Assisted Closure (VAC) therapy will stimulate the development of angiogenesis in the subjacent tissues underlying and surrounding the wound base to a greater degree than can be achieved with standard wound healing efforts.
Study Background: The presence of tissue ischemia and hypoxia due to compromised arterial blood flow is a primary factor compromising wound healing. Wounds that are compromised by impaired angiogenesis will have poor blood supply and are unlikely to progress through the stages of healing in an orderly and timely fashion. The VAC is a subatmospheric pressure system utilizing medical grade polyurethane foam wound dressing that is fitted at the bedside to the appropriate size for each patient's wound, and then covered with an adhesive drape to create an airtight seal. The VAC enhances wound healing by stimulating granulation tissue. The etiology of this granulation is thought to be increased blood flow to the wound base and stimulation of angiogenesis, but the physiologic tissue changes due to VAC therapy that lead to the development of angiogenesis has not been proven or documented. A recent clinical observation demonstrated angiographic evidence of subadjacent angiogenesis in a patient with a wound that was managed successfully with VAC therapy. However, to date no randomized controlled trials have been performed to document wound angiogenesis. We propose that subatmospheric pressure dressings applied to lower extremity ulcers will promote angiogenesis more effectively than moist wound therapy dressings.

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INTERACTION BETWEEN ENDOTHELIAL CELLS AND MYOFIBROBLASTS DURING WOUND HEALING

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Hypertrophic scars are a resultant of a fibroproliferative disorder observed at the time of the wound healing of large surface, like burns. In normal wound, the process of healing begins with the invasion in the wound of microvascular cells and by the migration of myofibroblasts to form the granulation tissue. There, myofibroblasts play an important role since they synthesize the extracellular matrix and are responsible for the contraction of the wound. At the end of the healing process, the myofibroblasts disappear by apoptosis via unknown stimuli. In the case of hypertrophics scars, there is persistence of a high density of cells and collagen in comparison with normal granulation tissue. Scientists associate this disorder with a problem in the regulation of apoptosis. Our hypothesis is that endothelial cells play an important role in the persistence of myofibroblasts in wound healing. We have compared the apoptotic rates of human myofibroblasts from normal wounds (Wmyo) with those from hypertrophic scars (Hmyo) when exposed with supernatants from microvascular cells isolated from normal skins (CEMV) or hypertrophic scars (CEMVH). The first results showed a significative reduction of apoptosis for Hmyo while Wmyo were not found to respond to the microvascular supernatant. There were, however, no difference in the apoptotic rates of both populations of cells when exposed to CEMV or CEMVH supernatants. These results suggest that endothelial cells secrete one or many factors that inhibit Hmyo apoptosis, thus preventing the disappearance of those cells and inducing the formation of hypertrophic scars. We hope that a better understanding of the mechanisms implicated in the formation of hypertrophic scars will open the way to new treatments.

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LASER SPECKLE PERFUSION IMAGING OF WOUND HEALING IN A PORCINE MODEL

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A Laser Speckle Perfusion Imaging (LSPI) system has been developed to achieve rapid, high-resolution, noninvasive imaging of tissue blood flow. This study was designed to evaluate LSPI for monitoring dermal blood flow during the wound healing process in a well-established porcine model. Full-thickness excisional wounds (2×2 cm) were created on the dorsal skin of 11 juvenile female red Duroc pigs. The skin wounds were imaged weekly with the LSPI system until day 49 postwounding. Blood flow values for the wounds were normalized using the values from the surrounding healthy skin to account for variations in systemic perfusion from one scan date to the next. An average normalized perfusion value was obtained from the scans of all animals for each time point. Wound perfusion at the time of reepithelialization reached ~165% of the surrounding skin ($p < 0.001$). This elevation in wound perfusion slowly and steadily decreased over time, until day 49 postwounding, at which time the values returned to normal. This trend toward decreased wound perfusion over time has previously been reported in human burn wounds using the LSPI instrument. Further, the LSPI data from this study and from other human studies correlates well with data generated using the more widely established Laser Doppler Imaging (LDI) technique. The faster scan time and higher resolution of the LSPI method provides a distinct clinical advantage, both in terms of patient comfort and for reliably matching perfusion characteristics to their associated anatomical features. The fast temporal response of the LSPI instrument could be used to monitor vascular responses to mechanical or pharmacological interventions to study dynamic vascular changes to damaged tissues. This study validates LSPI as a tool to measure blood flow in skin wounds and further supports the use of the juvenile female pig as a model of excisional wound healing, yielding results comparable to healing in humans.

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SUBSTANCE P INDUCE NITRIC OXIDE PRODUCTION IN HUMAN MICROVASCULAR ENDOTHELIAL CELLS

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Substance P(SP) modulates cytokine synthesis and adhesion molecule expression by inflammatory and endothelial cells in response to injury. A major proinflammatory effect includes nitric oxide(NO) production. Three nitric oxide synthase(NOS) isoforms include neuronal(nNOS), inducible(iNOS), and endothelial(eNOS). In this study, we determined which NOS enzymes mediate SP-induced NO upregulation in endothelial cells. Confluent human microvascular endothelial cells (HMECs) were treated with SP(10^{-8} M) for 48 h with and without the nonspecific NOS inhibitor N-nitro l-arginine methyl ester (L-NAME; 4 uM) or specific inhibitors against nNOS (L-thiocitrulline;60 nM), iNOS (n-3-aminomethyl benzyl acetamidine;7 nM), eNOS (N-5-1-iminoethyl-L-ornithine; 0.5 uM). Inhibitors were added for 3 h prior to and continued throughout SP stimulation. Nitrate/nitrite levels were measured in cell extracts using Greiss reagents and standardized with protein concentration for each sample. Results are expressed as uM nitrate/mg protein. Statistical analysis was determined using ANOVA. Result are expressed as mean ± sd; $p < 0.05$ was considered statistically significant. SP effectively elevated nitrate levels (2.96 uM/mg protein;p 0.05) compared to control treatment (2.1 uM/mg protein). All NOS inhibitors showed some inhibitory effect on substance P induced NO production. However, only L-NAME (1.9 uM/mg protein) and the specific eNOS inhibitor (2.32 uM/mg protein) demonstrated a significant decrease ($p < 0.05$.) Our data suggest that SP up-regulation of NO in HMECs is primarily mediated by eNOS, but that other NOS enzymes may also contribute. Further studies are warranted to better understand the role of NOS in SP signaling.

BIOENGINEERING POSTERS

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THE TREATMENT OF VENOUS LEG ULCERATIONS WITH A NOVEL FETAL BOVINE DERMAL COLLAGEN MATRIX WITH A COMPARISON TO PRECLINICAL ANIMAL STUDIES

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Background: Preclinical animal studies with a fetal bovine dermal collagen matrix suggest that fibroblasts quickly populate the material. At the same time there is a rapid ingrowth of new blood vessels. In short, the matrix quickly becomes living tissue. Experience with bioengineered skin constructs and porcine small intestinal submucosal products suggest that the application of a scaffold or matrix may play a role in the healing of venous leg ulcerations in humans.
Methods: A fetal bovine dermal collagen matrix (Primatrix) was applied to patients with long-standing venous leg ulcerations. Histologic examination pre and post application with trichrome staining was employed to differentiate matrix from native collagen. Comparisons between animal and human ulcers were made grossly and histologically.
Results: In all patients there was rapid formation of granulation tissue with a decrease in the depth of the wound. This was followed by epithelialization and wound closure. The stimulatory effect persisted for approximately 1 month after which time a second matrix was applied. In contrast to the animal study the grafts exhibited "take" in only one of our patients. Histologic examination in both animals and humans suggests a stimulation of native collagen production as well as an initial incorporation of the Primatrix into the wound bed.
Conclusion: Primatrix has demonstrated efficacy in preclinical studies and in a series of patients with venous leg ulcerations. The product may function as a scaffold for the ingrowth of cells essential to wound healing. Further investigation in a randomized controlled study setting is recommended.

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PHOTOCHEMICAL TISSUE BONDING OF APLIGRAF TO SKIN

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Background: Photochemical tissue bonding (PTB) is a novel method for producing a strong tissue-tissue seal using photosensitization. PTB cross-links tissues without causing thermal damage, although the mechanism of action in skin is not completely understood. Apligraf, a bilayered tissue-engineered skin construct, is currently applied to chronic wounds and fixed in place by bolster, suture, staple, or glue. We postulated that PTB may have application for attaching Apligraf to skin.
Materials and Methods: An ex vivo model was used to characterize bonding of Apligraf and skin by PTB. Argon laser (514 nm) or IPL irradiation in the presence of the photosensitizing dye Rose Bengal was used to bond Apligraf and human cadaveric or porcine skin. Various outcome measurements were made to evaluate the efficacy and histological characteristics of the bonding.
Conclusions: Photochemical tissue bonding of Apligraf to human skin can be performed and further characterization is under way. Potential applications of PTB and Apligraf include the treatment of acute wounds, split thickness skin graft donor sites, burns, and debrided chronic wounds.

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Abstract removed by request of author.

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ADIPOSE TISSUE ENGINEERING BY HUMAN ADIPOSE-DERIVED STROMAL CELL SEEDED GELATIN SPONGES

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The large bulk of lost craniofacial soft tissue is mainly adipose tissue. Current surgical approaches following tumor resection and trauma have limitations. Adipose derived-stromal cells (ADSCs), also named as preadipocytes, have been demonstrated to be a good cell candidate for adipose tissue engineering. However, characteristics of scaffold played important role in a successful tissue engineered adipose tissue.
Objectives: The present study is to investigate whether in vitro adipogenesis could be induced both in a monolayer and 3-D by ADSCs.
Methods: Healthy adult ADSCs were isolated by liposuction from fat pads. After discarding the unattached cells, the ADSCs were cultured in DMEM supplemented with 10% FBS and 1% antibiotics until they reached confluence. After trypsinization, ADSCs were subcultured at a density of 10^5 cells/100 mm-dish as a first passage. The monolayer and 3-D adipogenesis were assessed after seeding 10^5 ADSCs into each well of 6-well plates and 5×10^6 ADSCs/ml into biodegradable collagen sponges, respectively, followed by replacement of basic medium with adipogenic one.
Results: In both monolayer and 3-D scaffolds, lipids containing adipocytes were observed 1 week after adipogenic stimulation, and further increased over the time. By contrast, no adipogenesis was observed in ADSCs cultured in basic medium.
Conclusions: Human adipose-derived stromal cells isolated from fat pads can readily be induced toward adipogenesis in vitro. The in vivo adipogenesis potential of ADSCs is being investigated.

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IN SITU TISSUE ENGINEERING WITH INTEGRA®-A NEW PARADIGM OF SURGICAL WOUND REPAIR

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The paradigms of surgical wound closure are direct repair, grafts, and flaps. The core subject of plastic surgery, these distinct modalities are a sophisticated art that can reliably close any healthy wound. The caveat is that ordinary surgery implicitly depends on competent wound healing. With pathological wounds, those due to vascular, hematological, immunopathic, and other ulcerogenic diseases, these modalities may not work. The operation is threatened if wound healing is retarded, and incisions and donor sites are subject to morbidity from active disease. Disease and risks may limit potential donor tissues, sustain inflammation and lysis, restrict circulation, or make the patient too ill for elaborate procedures.
Integra® collagen-chondroitin matrix has ideal properties for managing chronic pathological wounds: a high-grade artificial skin; survives disease and conditions where grafts die; performs the coverage duties of flaps without donor sites; minimizes nursing care; suppresses inflammation, wound healing, and scar; induces embryonic dermatogenesis. Risk free to the recipient, it is safe where disease and altered anatomy make conventional closure impossible or unsafe. Its indications, use, results, and conceptual basis make it a distinct fourth paradigm of surgical wound closure: in situ tissue engineering. Understanding when a flap should but cannot be used is to understand when Integra should be used.
Study: Using a consistent set of indications and management scheme, Integra was used for chronic pathological wounds in 111 patients (158 individual ulcers, 166 instances of exposed anatomical structures, diverse diagnoses). Success rate was 92% of patients healed; 90% of open structures healed. Inpatient services were nearly eliminated.
Integra is not an alternative to conventional repair, grafts, and flaps. As a method of tissue engineering, it is a new and equal paradigm of care with its own indications and contraindications, and a superior safety and success profile. Repair, grafts, and flaps, which require wound healing competency, are best suited for healthy and acute wounds. For many chronic and pathological wounds, in situ matrix-guided histogenesis is the best method, and surgeons must begin working this into their practices.

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A NEW METHOD TO EVALUATE THE BIOMECHANICAL PROPERTIES OF SKIN IN A PORCINE MODEL

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Aims: A method to test the axial and transverse tensile properties of skin was developed in order to improve our understanding of the mechanical behavior of skin, and how it changes with the scarring response. This experimental technique was used to investigate the bidirectional mechanics of skin in the juvenile Yorkshire pig model.
Methods: Skin samples were taken upon sacrifice, and all subcutaneous tissue was carefully removed via dissection. Dumbbell-shaped specimens were obtained from the skin using a custom designed stainless steel punch, to provide consistent geometry, and to avoid stress concentrations that can result from specimen gripping. Samples were taken in the axial (cranial-caudal) and transverse (dorsal-ventral) directions. Specimens were secured in an INSTRON universal test machine with serrated soft tissue grips, elongated to a desired preload, subjected to a 10% elongation and held for a 4-min isometric period. They were then returned to the original length and failed in tension at constant velocity.
Results: This method of sample preparation indicated high repeatability in specimen geometry, showing standard deviations of 2% and 3% in gauge length and width, respectively ($N = 12$). Furthermore, no slippage was observed at the skin/grip interface, and all failures occurred within the specimen gauge length.
Conclusions: This technique shows great promise for evaluating the viscoelastic and failure properties of skin, scar tissue, and pathologic scarring. Combining this biomechanical method with our parallel studies in molecular biology and biochemistry can likely produce correlations between constituent material organization and the mechanical properties of the tissue, as well as provide valuable information for the design of bioengineered tissue constructs.

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MIST-ASSISTED HEALING: THE USE OF MIST™ ULTRASOUND FOR WOUND BED PREPARATION PRIOR TO THE USE OF BIOENGINEERED TISSUE (APLIGRAF®)

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The understanding of bioengineered tissue has evolved over the past 5 years. Now considered "cell therapy," bioengineered tissue can provide a nonhealing wound with the appropriate complement of cytokines, growth factors, and healthy cells to promote the patient's healing process. As clinicians have gained experience with Apligraf*, the process of wound bed preparation has proven to be a critical step in order to achieve optimal outcomes with the tissue construct. Recently, the investigators have evaluated a novel device, MIST† ultrasound , recently FDA cleared for the cleansing and debriding of wounds. A subgroup from a total of 23 patients in a research trial using MIST therapy for nonhealing wounds received an Apligraf as part of their treatment regimen. The bioengineered tissue remained intact for a full 4 weeks postapplication and the appearance of the graft was quite different than our prior experience with the product. On removal of the wound/graft eschar, patients had achieved complete epithelialization. This report describes the patients who underwent this combination of technologies along with wound photographs, measurements and patient histories. As we learn more about the biochemistry of healing we are able to "look" at how we combine and use advanced wound care technologies.

Acknowledgments: This study was supported from a grant from Celleration Inc.
Product notation: * Apligraf® is a registered trademark of Novartis, Inc., East Hanover, NJ © 2004 Organogenesis, Inc., Canton, MA † MIST™ ultrasound is a registered trademark of Celleration Inc., Eden Prairie, MN.

BIOMATERIALS POSTERS

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ALBUMIN DISPLACEMENT OF OLEIC ACID FROM WOUND DRESSINGS PROMOTES AN ELASTASE-LOWERING EFFECT IN CHRONIC WOUND FLUID

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The potential to lower destructively high levels of elastase found in chronic wounds with oleic acid-treated cotton wound dressings was assessed and compared with two types of oleic acid-treated occlusive wound dressings. The ability of albumin to bind oleic acid and transfer elastase inhibitory activity from the dressing material to elastase in solution was examined. Cotton, hydrogel, and hydrocolloid wound dressings were treated with oleic acid and tested for their ability to lower elastase activity. Oleic acid–treated cotton gauzes, hydrogels, and hydrocolloid dressings were found to release oleic acid in the presence of albumin in sufficient quantities to inhibit elastase activity. The order of elastase lowering activity with the three oleic acid-formulated wound dressings was cotton gauze > hydrogel > hydrocolloid. Elastase inhibition by oleic acid displaced from gauze followed a dose response profile. In contrast Cathepsin G, when displaced by albumin, was inhibited within a narrow range of oleic acid formulations. The effect of albumin levels representative of the chronic wound on displacement of oleic acid was determined. Solubilization of cotton bound oleic acid by albumin was found to be most effective at 4% albumin. However, there was sufficient solubilization of oleic acid at the 2% albumin levels found in chronic wound fluid to achieve a significant elastase-lowering effect. Albumin promoted solubilization of oleic acid from cotton with a solubility threshold of 27 mg/g cotton gauze. Equivalent lowering of elastase activity was found with 1%, 2%, and 4% albumin concentrations with oleic acid on cotton at 128 mg oleic acid/gram of cotton.

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APLIGRAF HEALS A RECALCITRANT PRESSURE ULCER OF 28 YEARS DURATION

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Background: Apligraf, a bioengineered living skin construct, has been shown to accelerate the healing of venous leg and diabetic foot ulcerations. It appears to function as cell-based therapy (e.g., delivering growth factors), rather than as a true graft. Given this mechanism of action, its use could be expanded to areas in which traditional skin grafting is not routinely employed.
Methods: A 64-year-old wheel-chairbound Caucasian male presented to the wound clinic with a grade III left trochanteric pressure ulcer which had been present since 1976. He had been seen by numerous physicians and treated with debridement, topical antimicrobials, negative pressure therapy, growth factors, and a variety of dressings. We spent several months preparing the wound bed: maintaining adequate moisture balance, performing serial debridement, utilizing offloading surfaces and reducing the bacterial burden. His nutritional status was optimized. The wound bed developed a healthy granulating base but failed to close.
Results: A single unit of Apligraf was meshed at a 1:1.5 ratio and applied to the wound. It was fixed in place using a nonstick silicone dressing, foam, and a self-adhesive covering. The dressing was changed weekly in the wound clinic. The initial response was a decrease in wound depth followed by steady epithelialization. Complete closure occurred 17 weeks after grafting. The wound has not recurred.
Conclusion: This single case study suggests that bilayered cell therapy may have application in difficult wounds, such as pressure ulcerations. Further study into the efficacy of Apligraf in pressure ulcerations is ongoing.

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CAN A TOPICAL ANTIMICROBIAL AGENT PENETRATE A BI-LAYERED CELL THERAPY AND BE EFFECTIVE AGAINST METHICILLIN-RESISTANT STAPHYLOCOCCUS AUREUS?

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Background: Bilayered cell therapy (BLCT) is a significant advancement in the field of wound healing. BLCT expresses multiple growth factors found in normal skin, and provides a biologically active matrix in chronic wounds. Antibiotic-resistant bacterium continues to be a major problem in chronic wounds. The use of appropriate topical antimicrobial agents could be one of the first steps in prevention of wound infection, although their use with BLCT is limited due to reported toxicity to cultured keratinocytes.
Objectives: This study was designed to see whether a topical antimicrobial agent is able to permeate through the BLCT and inhibits the growth of Methicillin-Resistant Staphylococcus Aureus (MRSA). Mupirocin was used because of its lack of toxicity to cultured keratinocytes.
Methods: MRSA (ATCC# 33591) strain was used for these studies. The entire surface of blood agar plates were covered with a high inoclum of MRSA. BLCT specimens were placed on the agar plates and mupirocin was then applied on the top surface. As a positive control, mupirocin was applied directly on the agar plate, while BLCT specimens alone were used as negative control. The plates were subsequently incubated for 24 hours after which the zones of inhibition were assessed.
Results: Placing mupirocin on top of BLCT gave a 41.9 ± 17.2 mm inhibition zone diameter. Although it was smaller than the inhibition zone diameter produced by direct administration of mupirocin (26.3 ± 9.4 mm), this difference was not statistically significant. BLCT by itself did not create a zone of inhibition.
Conclusion: Mupirocin is able to permeate BLCT and inhibit the bacterial growth underneath it. This study may have important clinical implications.

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SYNTHETIC, CYCLODEXTRIN-BASED POLYMER MATRIX FOR LOCAL REGIONAL DRUG DELIVERY

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Various matrices containing both synthetic or natural polymers are currently used for local regional drug delivery to a number of disease locations such as chronic cutaneous wounds, joints, bone, muscle, and nerves. Insert Therapeutics has developed a novel synthetic matrix which consists of a copolymer of β-cyclodextrin and polyethylene-glycol (PEG). Addition of di- or multifunctionalized adamantane-PEG molecules results in noncovalent cross-linking through inclusion complex formation. The resulting hydrogel showed rheological characteristics amenable to topical application or local-regional injection into a variety of tissues (Bellocq et al. (2004) Bioconjug Chem 15(6): 1201–1211).

This matrix was shown to be biocompatible, allowing for cellular growth and migration in vitro. It also allowed for efficient delivery of an adenovirus gene therapy vector to dermal fibroblast cells. In vivo, the matrix demonstrated efficient delivery of biotherapeutics such as recombinant adenovirus and non-viral gene therapy vectors after intradermal injection. The matrix was well tolerated and was as efficient as collagen in promoting wound healing when an adenoviral gene therapy vector expressing PDGF-bb was delivered to cutaneous wounds of diabetic mice.

The matrix can additionally be modified to incorporate therapeutic small molecules, peptides, or proteins, either through inclusion complex formation or chemical linkage. Using biodegradable linker chemistry, the release rate of covalently attached molecules can be controlled. The cyclodextrin-PEG matrix is therefore an attractive alternative to existing matrices that is biocompatible, biodegradable, tunable with regard to its physicochemical properties, and can be designed to deliver multiple therapeutic agents to a variety of tissues in a controlled fashion.

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AN ADVANCED WOUND DRESSING WITH SUPERABSORBENT, MICROBICIDAL, AND HEMOSTATIC PROPERTIES

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Bacterial growth within extended use wound dressings remains a problem. We have developed a superabsorbent, microbicidal dressing based on the permanent attachment of a cationic polymer (the quaternary ammonium compound diallyl dimethyl ammonium chloride, DADMAC) onto a range of physical substrates. Treated gauze (Sof-Wick™) absorbed 50x its weight in saline and reduced bacterial, viral and fungal growth by six logs in vitro (AATCC method 100), with no zone of inhibition or extractables, as shown in Figure 1 below. Bacterial kill occurs within minutes, and remains effective in 10% serum. Good hemostatic properties were demonstrated for treated gauze wound dressing using both a rat liver laceration model, and a renal artery transection model, showing equivalent efficacy to approved haemorrhage control dressings (Avitene, Surgicel, and Gel Foam) that do not claim microbicidal activity. Treated gauze passed cytotoxicity testing using rabbit eye and skin tests, as well as guinea pig dermal sensitization tests.

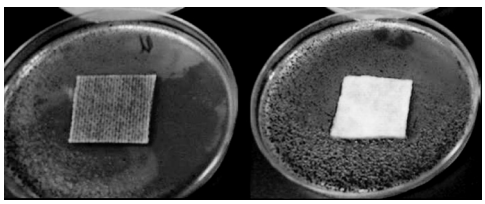

Figure 1: Gauze sponges inoculated with E. Coli and stained for bacterial presence. Left is control; right is NIMBUS treated. Note no zone of inhibition around treated gauze.

Acknowledgment: This research was supported by QuickMed Technologies.

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RETAINING THE VIABILITY OF HUMAN AMNIOTIC MEMBRANE FOR USE IN BURN WOUND COVERAGE

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Introduction: Currently freeze-dried, gamma-sterilized, or glycerol-preserved amniotic membranes are widely used. However, it is not clear whether this devitalized state is the optimal application form. Therefore within this study the ideal condition for midterm storage of human amniotic membranes was assessed to ensure the availability of vital amniotic membranes, in particular for burn wounds.

Methods and Materials: For this purpose mothers were serologically tested and term placentae were collected and washed. After the amniotic membrane was peeled off and further washed, biopsies were taken for microbiological testing and various storage experiments (different media and temperatures).

- cell culture medium, 37 °C
- glycerol, 4 °C
- 10% DMSO, −80 °C

Viability of fresh and stored amniotic membranes was determined with the MTT-based EZ4U- Assay (Biomedica, Vienna, Austria).

Results and Discussion: Best results were obtained while storing the membranes in cell culture medium at 37 °C, whereas storage in glycerol at 4 °C resulted in cell death within the first 4 days.

To our knowledge this is the first study investigating the viability of amniotic membrane under different storage conditions. The influence on wound healing is currently under investigation.

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RECONSTRUCTION OF RECURRENT FIRST DORSAL WEB SPACE ONCOLOGIC DEFECTS WITH ACELLULAR DERMIS

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Introduction: Open wounds following oncologic reresection of skin cancer of the hand can be problematic from a soft tissue reconstructive perspective. First, these wounds must often await definitive coverage until permanent negative margin clearance is obtained. This results in the morbidity of chronic painful dressing changes and possible infection. Secondly, for defects of the first web space, the reconstructive options are often limited to skin grafting which can yield functional hazards with contracted healing. Acellular dermis has been proposed as a biomechanical scaffold for subsequent skin grafting.

Hypothesis: We proposed to use acellular dermis for wounds stemming from reresection of skin cancers of the first web space to:

1) obviate dressing changes until permanent histology was confirmed
2) provide a soft tissue platform that enhances subsequent skin graft take and healing and ameliorates potential graft contracture

Methods: The resected wound area of two patients, 11×5 and 5×4 cm in size, were transiently covered with acellular dermis in the first stage. After confirmation of final histologic clearance of tumor at the margins, split thickness skin graft was placed on the granulating wound bed. Subsequent clinical examination and DASH questionnaires were used to assess outcome.

Results: After 6 months, wounds had healed with good cosmetic result. No contracture of the web space was noted. Average DASH score was 20.5 and no recurrences were noted.

Conclusion: Our observations suggest that acellular dermis is a useful adjunct for covering wounds of the hand, especially for functionally important locations such as the first dorsal web space.

WOUND INFECTION POSTERS

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EXPRESSION OF CATHELICIDIN (LL-37) IN THERMAL INJURY

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Sepsis remains a common and serious complication of major burn injury and currently accounts for over 54% of deaths in burn patients. Burns have been associated with high levels of circulating proinflammatory cytokines and immunosuppression which promotes systemic inflammatory response syndrome (SIRS) and sepsis, for which no effective treatment is currently available. Defensins and cathelicidins, a family of cationic naturally occurring antimicrobial peptides, are considered important components of the innate immune system as they play a major role in the body's defense by inhibiting several bacteria, fungi and enveloped viruses. Our prior studies using RT-PCR and fluorescence deconvolution microscopy suggest decreased expression of human β defensin 2 (HBD2) in burn wounds. In this study we have characterized cathelicidin (LL-37) protein levels using in representative skin samples of deep partial and full thickness burns and in unburned skin. Our results show that in unburned skin, the majority of LL-37 was located in the malphigian layer and was concentrated on the stratum corneum as well as colocalized with sweat ducts. In burned skin, in which the epidermis was destroyed, this pattern of LL-37 was absent. The surviving dermal and subcutaneous layers revealed LL-37 presence in very high concentrations colocalized with sweat duct epithelia. The result of these studies will contribute to an understanding of the role of antimicrobial peptides in the pathophysiology of burn injury, associated immunosuppression, and sepsis.

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MODULATION OF GENE EXPRESSION BY NORLEU^{3}A(1–7) IN DIABETIC WOUNDS

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Neuropathic ulcers represent a significant cause of morbitity for diabetics in the United States. Diabetic wounds are notoriously difficult to resolve. Treatment options are limited due in part to the lack of knowledge about the molecular events that regulate successful resolution of wounds. To identify differences between normal and diabetic gene regulation in wounds, we examined the level of expression of 12,000 genes in granulation tissue and wound-edge epithelium in normal animals and in an experimental model of diabetes following induction of full thickness skin injuries. Full thickness injuries in diabetic mice were characterized in part by altered regulation of structural genes and genes that regulate energy metabolism and utilization. The energy cycle contributes backbone structures for the synthesis of a variety of amino acids, the molecular building blocks essential for wound repair. A direct consequence of a diminished ability to efficiently exploit energy supplies would be decreased availability of regulatory and structural proteins necessary for efficient healing in diabetic wounds. We have successfully enhanced healing in diabetic wounds by treatment with the angiotensin II peptide NorLeu3A(1–7). NorLeu3A(1–7) accelerates healing of full thickness injuries in diabetic mice. While NorLeu3A(1–7) enhances healing by accelerating collagen deposition and reepithelialization, gene array studies show that NorLeu3A(1–7) acts locally within the wound site by altering gene expression within the granulation tissue. Specifically at day 7 after injury, NorLeu3A(1–7) reduced the expression of MMP genes and increase the expression of genes involved in energy metabolism.

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ANTIMICROBIAL ACTIVITY OF SYNTHETIC CATIONIC STEROIDS ON BURN WOUND PATHOGENS

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Sepsis remains the single most important cause for multiple organ failure and death following burn injury for which no effective treatment is currently available. Burn injury is associated with immunosuppression which promotes sepsis. Studies from our laboratory have demonstrated that levels of the antimicrobial peptide (AMP), human β-defensin-2, was significantly decreased in burned skin epithelia, absent in burn blister fluid and poorly expressed in lung during inhalation injury. We hypothesize that 1) deficient antimicrobial peptides in burn wound promote progressive colonization of pathogens in burn wounds and 2) replacing active peptide components will restore the antimicrobial activity in burn wounds. We have synthesized several cationic steroid antimicrobials (CSAs) which mimic naturally occurring AMPs functionally. In vitro experiments using one of these synthetic drugs with locally isolated common burn pathogen P. aeruginosa and S aureus demonstrate MIC values of $2.0 \pm 0.3\,\mu g/ml$ and $0.4 \pm 0.1\,\mu g/ml$, respectively, indicating effectiveness of these compounds against burn pathogens. These drugs were tested for cytotoxicity on cultured human keratinocytes using MTT cell viability assay. Results indicate that these drugs are nontoxic to cultured keratinocytes at the level required for bacterial killing. Since theses drugs are membrane active compounds it will be hard for bacteria to develop resistance and there is a great potential to be developed for clinical treatment.

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OPEN LABEL PILOT STUDY OF PROLONGED RELEASE NANOCRYSTALLINE SILVER DRESSING (ACTICOAT 7): REDUCTION OF BACTERIAL BURDEN TREATMENT IN THE TREATMENT OF CHRONIC VENOUS LEG ULCERS

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In persons with venous ulcers and an absence of arterial disease, high compression as exemplified by the four layer bandage (Profore) has been demonstrated to be effective by a meta-analysis of existing studies. Despite optimized compression, some venous ulcers do not heal at the expected rate and persistent inflammation or infection may delay or prevent healing. We report the results of a case series that demonstrates an ionized silver dressing with prolonged release of nanocrystalline crystals (Acticoat 7) can decrease bacterial burden and accelerate healing in venous ulcers not healing at the expected rate. Patients with venous ulcers were treated with a prolonged release absorptive nanocrystalline silver dressing (Acticoat 7) under a four layer bandage (Profore) for 12 weeks, or until healing. The primary efficacy objective measured the effect of the silver dressing on the wound microflora. Biopsies of the wounds were taken at baseline and after treatment with the silver dressing (Acticoat 7) and were analyzed for the bacterial species and number of bacteria present. In addition, serum silver levels were assessed at baseline, weeks 4, 8 and week 12 or final assessment. A total of 15 patients (9 male, and 6 female) were enrolled into the study. The median age was 63 years (range 30–83 years). The median duration of current ulceration was 17.3 weeks (range 4 weeks to 11 years) and the median ulcer area was $4.8\,cm^2$ (range $1.8–43.9\,cm^2$). The median exposure to Acticoat 7 was 82 days (range 8–86 days). There was a statistically significant reduction ($p = 0.0114$) in the $\log_{10}$ (total bacterial count) between the baseline and final biopsies (median 4.48 and 3.00, respectively). Four patients healed, 8 patients continued to the end of the 12-week study period and three patients were discontinued early. Of those patients who did not heal, 4 had more than a 94% reduction in wound area by the end of the 12-week study period. For all patients, the median percentage reduction in ulcer area was 94.4% and the median final ulcer area was $0.4\,cm^2$. Statistical analysis showed a significant increase ($p = 0.054$) in serum silver concentration during the treatment period. At baseline, prior to the silver dressing treatment (Acticoat 7), the median silver concentration was $0.3\,\mu g/L$ (range: 0.20–1.90 μg/L). The median within patient change from baseline serum silver concentration was an increase of $0.15\,\mu g/L$ (range: $-0.3–2.8\,\mu g/L$). Although this small increase was observed it was not considered clinically significant. This study illustrates the use of prolonged release nanocrystalline silver dressing to decrease the bacterial burden of chronic venous ulcers not healing at the expected rate.

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POTENTIAL USE OF NITRIC OXIDE GAS AS AN ANTIMICROBIAL AGENT IN CUTANEOUS WOUND INFECTION

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Despite recent advances in wound care, chronic infected ulcers still remain a huge challenge to the modern society. Nitric oxide has been identified as a mediator for important biological processes such as vasodilation, antimicrobial, and wound repair. The aim of the present study was to explore the antimicrobial properties of exogenous nitric oxide gas (gNO) as well as its effect on skin cells in vitro. To test this, a specialized gNO chamber was designed to expose *S. aureus* and *P. aeruginosa* in saline continuously to 200 ppm gNO or air. A colony count was obtained following gNO treatment. Furthermore, fibroblast-populated collagen gel, keratinocytes, and endothelial cells were cultured in DMEM, KSFM, and M199, respectively. Endothelial migration was tested by a 3D Matrigel tubule formation assay. Fibroblast matrix production and keratinocyte differentiation were monitored by mRNA expression of procollagen type I, interstitial collagenase, and involucrin. All cells were exposed to 200 ppm gNO for 8 hours a day for 3 consecutive days. A MTT assay was used to observe cell proliferation. The results revealed 100% bacterial kill following 4 hour exposure to 200 ppm gNO. Interestingly this dose did not exhibit any significant toxic effect on fibroblast, keratinocyte, and endothelial proliferation. Dermal fibroblasts migration out of the collagen gel was not affected by gNO treatment when compared to the control. The keratinocytes in both groups were able to express involucrin, an indicator of cell's ability to differentiate. The migration and tube formation by endothelial cells within the matrigel was not compromised following gNO treatment. In conclusion, the present study provided evidence for potential application of gNO for reducing bacterial burden in chronic infected wounds without compromising re-epithilialization, proliferation, and angiogenesis in the wound healing process.

Acknowledgment: This study was funded by PulmoNOx Medical Inc.

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EFFECTS OF LOW-FREQUENCY ULTRASOUND APPLIED IN VITRO TO HIGHLY ANTIBIOTIC-RESISTANT ACINETOBACTER ISOLATES RECOVERED FROM SOLDIERS RETURNING FROM IRAQ

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Abstract Brooke Army Medical Center isolated 25 highly antibiotic-resistant ***Acinetobacter ssp***. (primarily ***A. baumannii***) from wounded soldiers returning from Iraq. Concern about effective treatment of these organisms led our institution to begin investigating low-frequency ultrasound (LFU) as a method of increasing the effectiveness of antibiotics on ***A.baumannii*** in wound management. Studies have suggested that LFU applied in conjunction with antibiotics may increase their overall effectiveness. We hypothesize that combining antibiotics with LFU may be an effective method of wound management and that this combination may be synergistic in its overall effect. In this initial work, we wanted to determine if sonocation would have an effect on our organism of interest, ***A. baumannii***. We selected several organisms, both gram positive and gram negative, that have been shown to be killed by sonocation (***E. coli, S. aureus***, and ***S. pyogenes***) and added three highly resistant ***A. baumannii*** isolates. Bacterial death was measured by both colony counts after 24 hours of growth and acridine orange staining using a standard protocol.
Colony counts were significantly reduced by sonocation. Furthermore, ***A. baumannii*** colony counts were also greatly reduced by sonocation. Actual cell destruction was also visualized using acridine orange staining. Our data support the assertion that sonocation has an antibacterial effect on some bacteria, including ***A. baumannii***. Our next step is to add antimicrobial agents and determine if their effectiveness can be increased by sonocation.

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THE EFFECT OF VARIOUS ANTIMICROBIAL AGENTS ON *ACINETOBACTER BAUMANNII*: AN IN VITRO EVALUATION

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Background: *Acinetobacter baumannii* has recently emerged as an important hospital-acquired pathogen, especially in surgery, burn, and intensive care units. Due to its ability to develop resistance to antimicrobials, wound infection with *A. baumannii* is difficult to treat, and can lead to septicemia and even death. Use of appropriate topical antimicrobial agents in these circumstances could be one of the first steps in prevention of *A. baumannii* wound infection.
Objectives: In this study, we will discuss the in vitro effects of seven common topical antimicrobial creams and dressings on *A. baumannii*.
Methods: A. Baumannii ATCC# 6919 was subjected to sensitivity tests against mupirocin, silver sulfadiazine, mafenide acetate, a double antibiotic combination of polymyxin and bacitracin, a triple antibiotic combination of Neomycin, bacitracin and polymyxin, and two silver-containing dressings. Zones of inhibition were measured after 24 hours incubation period.
Results and Conclusion: Of the evaluated antimicrobial agents, mafenide acetate was the most efficacious followed by mupirocin, triple and double antibiotic combinations in decreasing order. The silver-containing dressings yielded a lesser zone of inhibition as compared to the previously mentioned, and no zone of inhibition was observed using silver sulfadiazine. Further in vivo studies on the effect of antimicrobial agents against *A. Baumannii* are necessary to substantiate these findings and determine the potential clinical relevance of these therapies.

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EFFECTS OF A SILVER HYDROFIBER® DRESSING ON THE QUANTITATIVE BACTERIAL BURDEN, REDUCTION IN ULCER SIZE AND EXUDATE OF CHRONIC NON-HEALING

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Often chronic wounds have an increased bacterial burden that can impair healing without the classical clinical signs of infection. Silver dressings may provide an alternative topical method to control bacterial burden.
The primary aim of this study was to evaluate the effect of 2–4 weeks therapy with the Silver Containing Hydrofiber® dressing on quantitative bacterial burden and clinical improvement in chronic wounds not healing at the expected rate.
This was a single centre, four-armed study which included a total of 30 patients with diabetic foot ulcers, leg ulcers, pressure ulcers and miscellaneous wounds that did not fit into any of the above categories. Patients had a baseline quantitative bacterial biopsy and this was repeated at weeks 2 to 4. The wound size was recorded along with a semi quantitative estimate of exudate and the periwound temperatures. Repeat measurements were performed at the follow-up visits and the decrease in wound size calculated. The underlying cause of the ulceration was be treated and corrected. This was followed by application of silver containing hydrofiber® dressing. There was a significant delay in healing of the leg ulcers associated with increased bacterial burden in the quantitative biopsy bacterial burden results at week 0 and healing at week 2. ($p = 0.01$). Other subgroups had a similar association that did not reach statistical significance. The presence of an increased exudate in the leg ulcers at week two was associated with delayed healing at week 4 ($p = 0.05$). There was also a significant increase in skin surface temperature of the surrounding skin with an increased quantitative bacterial biopsy of the deep wound compartment for venous, diabetic neurotrophic foot ulcers and pressure ulcers with p values of 0.05, 0.01 and 0.01 respectively. There was no significant decrease in exudate or increased healing of the wounds with the application of the silver hydrofiber dressing in this difficult to heal population. The population studied in this case series had increased bacterial burden in the deep compartment as measured with increased exudate and or an increased temperature of the periwound skin. These patients have an increased bacterial burden in the deep wound compartment that does not respond to topical ionized silver in the dressing studied.

CLINICAL TRIALS POSTERS

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INTERVAL ANALYSIS OF A RANDOMIZED, OPEN LABEL, MULTI-CENTER STUDY COMPARING APLIGRAF VS. STANDARD MULTILAYER COMPRESSION IN THE REDUCTION OF PAIN ASSOCIATED WITH VENOUS LEG ULCERATIONS

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Background: According to The American Pain Society, pain should be considered a fifth vital sign. Pain is a prominent feature of venous leg ulcerations (VLUs). Sixty-five percent of VLU patients complain of severe pain. In several multicenter clinical trials, Apligraf, a bioengineered living skin construct, has been shown to accelerate healing in venous leg ulcerations. Anecdotal clinical observations suggest that Apligraf treatment is associated with pain relief. A treatment modality which promotes healing and reduces pain would significantly improve the quality of life in patients with VLU.
Methods: VLU patients with pain scores greater than 5/10 on a numerical scale or requiring narcotic analgesia were randomized to receive either Apligraf and compression or compression alone. There was a 3-week "wash-out" period to ensure that pain was not due to other factors such as inadequate compression or infection. Patients were enrolled only after a negative quantitative biopsy. The intensity of pain was self-scored and a quality-of-life questionnaire was completed at each visit. Weekly wound area measurements were obtained with digital planimetry.
Results: Thus far in the enrollment period we have already seen a trend toward a significant reduction in pain in the Apligraf group. This pain relief occurs shortly after application indicating that a mechanism distinct from clinical healing. The Apligraf group also demonstrated acceleration in wound healing as well as a decreased time-to-complete closure.
Conclusion: Interval analysis of this multicenter trial suggests that Apligraf accelerates wound healing and reduces the pain associated with venous leg ulcerations.

Acknowledgment: Unrestricted Educational grant, Organogenesis Inc.

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MIST ULTRASOUND: THE RESULTS OF A PROSPECTIVE, NON-RANDOMIZED EFFICACY STUDY OF RECALCITRANT WOUNDS

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MIST ultrasound, a recently FDA-cleared noncontact ultrasound therapy, was utilized prospectively in an IRB-approved trial for the treatment of recalcitrant wounds of various etiologies. Twenty-nine wounds from 23 patients were studied. Inclusion criteria included wounds greater than 4 weeks duration with no evidence of clinical improvement (>15% area reduction) over the prior 2 weeks before enrollment. The wounds were required to be free from clinical signs of infection. After an initial debridement and wash-out period, patients were treated 3 times a week with MIST for 4 minute treatment applications. Dressing regimens were left up to the investigator as dictated by wound conditions. The primary endpoint is complete healing. Data will be presented looking at MIST-derived healing, as defined by complete healing using MIST therapy all the way to closure. MIST-assisted healing, as defined by MIST therapy for wound bed preparation followed by an alternative means of closure will also be presented. "Overall healing" defined by the combination of the two prior groups will complete the outcomes report, as this reflects a more "real-world" clinical application for the technology. Wound dynamics, Kaplan Meier survival plots and laser Doppler imaging estimations of angiogenesis will all be presented.

Acknowledgment: This study was supported with a grant from Celleration Inc., Eden Prairie, MN.

GENE THERAPY POSTERS

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INVOLVEMENT OF THE HUMAN ANTIMICROBIAL PEPTIDE LL-37 IN WOUND REPAIR

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Antimicrobial peptides are major components of the innate defense system that present microbicidal activity against gram positive and negative bacteria, yeast, and some enveloped viruses. The human cathelicidin LL-37 is expressed in skin upon wounding and, in addition to its role in antimicrobial defense, it has also been previously demonstrated that this peptide might be involved in reepithelization. We have generated an adenoviral vector containing the complete sequence of LL-37 along with an Ires-GFP expression cassette. The efficacy of this vector was probed by testing the conditioned medium from adenoviral transduced keratinocytes in preventing bacterial growth. We have studied the in vitro effects of LL-37 on HK migration and proliferation. In addition, we have efficiently transduced human skin grafts (in immunodeficient mice) using an in vivo gene transfer approach and we are currently testing the involvement of this peptide in wound repair.

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IN VIVO ADENOVIRAL GENE TRANSFER OF SPARC IN A SKIN-HUMANIZED MOUSE WOUND HEALING MODEL

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SPARC (secreted protein, acidic and rich in cysteine), a matricellular glycoprotein, modulates the interactions of cells with the extracellular matrix. Studies in null-mice revealed a role of SPARC in wound healing. Here we examined the effect of SPARC in a skin-humanized mouse wound healing model. This model is based in the regeneration of human skin onto the back of nude mice by transplantation of a dermo-epidermal equivalent. The regenerated human skin was excisionally wounded with biopsy punches. At the moment of wounding an adenoviral vector encoding the cDNA for SPARC was intradermally injected. We are currently assessing the effects of in vivo gene transfer of SPARC at the wound site in the healing process. Criticals events of wound healing including reepithelialization, regeneration of dermoepidermal junction and dermal remodeling were studied at different time points postwounding.

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LENTIVIRAL GENE THERAPY WITH THE GENE FOR PDGF-B IMPROVES DIABETIC WOUND HEALING IN AN ANIMAL MODEL

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Diabetic wounds are characterized by disruption of the normal wound repair responses, particularly at the inflammatory and proliferative stages. Inadequate levels of growth factors, such as platelet-derived growth factor (PDGF), play a significant role in the impaired healing of these wounds. PDGF is a potent mitogen and chemotactic agent, and is released from platelet alpha-granules during the acute inflammatory phase of wound healing. It has been shown to promote the deposition of collagen and granulation tissue, and improves healing in diabetic animals. Although previous studies have shown some success with topically applied PDGF, results in clinical practice have been moderate. In this study, we investigated whether gene therapy with lentiviral vectors carrying the gene for PDGF can improve wound healing in an animal model. Full thickness 2 cm × 2 cm dermal wounds were created on the dorsae of genetically diabetic db/db mice. Lentiviral vectors containing the human PDGF-beta gene (lenti-PDGF) were injected into the wound margins and along the wound base. Control experiments were performed using phosphate-buffered saline (PBS) injections. Mice were sacrificed after 21 days, and wound tissues were harvested for histological analyses. Lenti-PDGF-treated animals demonstrated significantly smaller residual epithelial gaps compared to PBS controls (0.77 +/− 0.07 cm vs. 1.22 +/− 0.30 cm; $P < 0.001$), as well as a greater degree of wound closure (85 +/− 3% vs. 57 +/− 22%; $P < 0.05$). Lentiviral gene therapy with PDGF is effective in promoting wound healing in genetically diabetic animals, and warrants further investigation.

Supported by a grant from the American Diabetes Association

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A "TRAFFIC CONTROL" ROLE FOR TGF-BETA IN SKIN CELL MOTILITY DURING WOUND HEALING

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Skin wound healing requires temporally and spatially coordinated motility of multiple skin cell types. The limited success of current skin wound care is due to lack of understanding about how the healing processes begin, progress, and complete. One of the most dramatic environmental changes in an acute wound is the transition from plasma to serum. The cells in the wound come into contact with serum, instead of a filtrate of plasma, for the first time. We report here that this transition differentially regulates the migration of various skin cell types. Plasma promotes dermal fibroblast and endothelia cell, but not keratinocyte and melanocyte, motility. In contrast, serum selectively promotes keratinocyte motility and inhibits fibroblast and endothelial cell motility. The key regulators are the increased transforming growth factor-beta (TGF-beta) in serum and the distinct TGF-beta receptor (I, II and III) profiles in different cell types. TGF-beta selectively inhibits fibroblast and endothelial cell, but not keratinocyte and melanocyte, migration. Neutralization of TGF-beta function in serum eliminates the differential effects between serum and plasma. Manipulations of the TGF-beta receptor profiles in the cells, especially TGF-beta receptor II and III, convert TGF-beta-insensitive cell types to sensitive cell types and vice versa, and their responses to plasma and serum. This is the first study that reveals a molecular mechanism for coordinating multiple skin cell-type motility in wounds.

GROWTH FACTORS POSTERS

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INFLUENCE OF EPIDERMAL CELLS IN HYPERTROPHIC SCARRING PATHOLOGY

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Hypertrophic scarring is a pathological process characterized by a fibroblastic hyperproliferation and by an excess of deposition of extracellular matrix components. Hypothesis of abnormalities in epidermal-dermal cross talk has been laid down to explain this pathology. To check this affirmation, we used a tissue-engineered model of self-assembly reconstructed skin in order to mimic interactions between dermal and epidermal cells in normal or pathological skin. We performed those skin equivalents with three dermal cell types : normal wound (Wmyo) or hypertrophic wound (Hmyo) myofibroblasts and normal skin fibroblasts (Fb). Epidermis was reconstructed with normal skin keratinocytes (NK), hypertrophic scars keratinocytes (HK), or without any keratinocytes. In the absence of epidermis, Hmyo formed a thicker dermis than Wmyo. When seeded with NK, the dermal thickness of Hmyo and Fb dermis was significantly reduced while that of Wmyo was increased. However, the presence of HK always induced thicker dermis formation than observed with NK. HK also increase the production of extracellular matrix and reduce its degradation in comparison with NK. In addition, HK have more influence on dermal cells proliferation than NK. In conclusion, HK may play a more important role on fibrosis development than NK. The keratinocytes have distinct secretory patterns depending on their skin source. Wmyo and Hmyo do not react the same way to the presence of keratinocytes. These observations strongly suggest that HK have a more important role on pathological fibrosis development by influencing dermal cells behavior.

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RETROVIRAL GENE THERAPY WITH THE GENE FOR PDGF-B PROMOTES WOUND HEALING IN DIABETIC MICE

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Platelet-derived growth factor (PDGF-B) is a potent cell mitogen and chemotactic signal involved in normal wound healing. PDGF-B is present at decreased levels in diabetic wounds and has been shown to improve wound healing when applied in a topical form. However, clinical results with topical PDGF in the treatment of diabetic wounds have been equivocal. In this study, we investigated whether application of PDGF-B via gene therapy can effectively promote wound healing in the diabetic environment in an animal model. Retroviral vectors carrying the gene for human PDGF-B were constructed using the LNCX virus and the G418 resistance gene for selection (LN-PDGF-B), and transduced into mouse dermal fibroblasts. LN-PDGF-B transduced fibroblasts were seeded into alginate hydrogel scaffolds at a concentration of 25×10^6 cells/implant and implanted into 2 cm × 2 cm full thickness excisional dermal wounds created on the dorsae of genetically diabetic db/db mice. At 21 days, animals treated with LN-PDGF-B transduced cells suspended in alginate hydrogel demonstrated a significantly smaller epithelial gap (0.97 +/− 0.34 cm) than animals receiving untransduced mouse dermal fibroblasts alone (1.44 +/− 0.37 cm; $P < 0.05$) or animals receiving dermal fibroblasts transduced with LNCX viral vector alone (1.40 +/− 0.20 cm; $P < 0.01$). These results suggest tissue-engineered retroviral gene therapy with PDGF-B specifically promotes diabetic wound healing and may yield advances in the effective treatment of diabetic wounds.

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DEPRESSED AND LONELY INDIVIDUALS EXPRESS LOWER EGF AND VEGF IN SALIVA

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Epidermal growth factor (EGF) and vascular endothelial growth factor (VEGF) are present in saliva and may impact wound healing within the oral cavity. Previous studies in our lab have demonstrated that psychosocial factors such as elevated depression scores are associated with delayed oral wound healing. The aim of this study was to determine the relationship between salivary levels of EGF and VEGF and depression and loneliness scores. Whole saliva was collected from 43 individuals in groups aged 18–35 years and 50+ years. On the same day, volunteers were asked to complete the Beck's Depression Inventory (BDI) and the UCLA loneliness scale. Salivary concentrations of EGF and VEGF were determined by standard ELISA. Mean concentrations of EGF and VEGF were 2860 pg/ml and 421 pg/ml, respectively. In the younger age group, significant negative correlations were found between scores on the BDI and UCLA indices and salivary levels of EGF. A median split, based upon BDI scores, similarly revealed that more depressed or lonely individuals exhibited significantly lower EGF levels. A similar trend was observed for VEGF. These preliminary results indicate that psychosocial factors such as depression and loneliness may be related to growth factor expression in saliva. This, in turn, may play a part in the delayed mucosal wound healing observed in depressed individuals.

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REGULATION OF TGF-β AND ITS SIGNALING COMPONENTS BY OXYGEN TENSION AND STEROIDS IN SKIN CELLS

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Inappropriate control of the expression and function of the components of the TGF-β signaling pathway has been implicated in impaired wound healing and abnormal scarring. Factors that have been shown to have profound effects on skin homeostasis and wound healing include steroids and oxygen tension. These agents may exert their effects (at least in part) on the skin and wound healing by modulating TGF-β action. To identify steroids and oxygen tension as mediators of TGF-β action in the skin, we examined the effects of hypoxia and glucocorticoids on the levels of active TGF-β and its signaling receptors and on the phosphorylation of its intracellular mediators, Smad 2 and Smad 3, in skin fibroblasts. Our results show that the active TGF-β levels, expression of types I and II receptors and phosphorylation of Smad2 and Smad3 are markedly up-regulated by 2 hrs of hypoxia, whereas all these parameters are significantly downregulated by 24 hrs of hypoxia. Dexamethasone treatment of 2 hrs, on the other hand, increased active TGF-β levels and TGF-β receptor expression with little effect on the levels of phosphorylated Smad2 and Smad3. However, dexamethasone treatment of 24 hrs downregulated the active TGF-β levels while maintaining the higher receptor expression, again with no significant effect on phosphorylated Smad2 and Smad3 levels. Interestingly, hypoxia had an additive effect on the steroid-induced downregulation of active TGF-β levels while counteracting the steroid-induced upregulation of TGF-β receptor expression. The upregulation at 2 hrs and the downregulation at 24 hrs of the components of TGF-β signaling pathway by hypoxia is consistent with the beneficial effect of initial hypoxia and the deleterious effect of sustained hypoxia, respectively, on wound healing. The synergestic and antagonistic effects of dexamethasone and hypoxia on the TGF-β signaling components emphasize the complex regulation of TGF-β signaling at multiple levels.

INFLAMMATION POSTERS

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THE IMPORTANCE OF THE REDUCTION IN LOCAL IRRITANT EFFECTS OF WOUND EXUDATES BY EFFECTIVE WOUND MANAGEMENT FOR THE PROGRESSION OF NONHEALING WOUNDS

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How wound dressings may act locally to aid chronic wound healing is poorly understood. Along with an increased understanding of the pathophysiology of nonhealing wounds such as venous leg ulcers and diabetic foot ulcers, and the realization that these ulcers exhibit a high degree of inflammation, models have been developed linking the disease etiology and the visible ulcer. Furthermore, these models have suggested ways in which present therapeutic regimens, such as compression, aid healing. However, little focus has been placed on understanding how locally applied wound dressings aid in the overall healing response in the context of elevated inflammation. Here, we propose that if wound dressings have the correct combination of novel materials and physical characteristics they may provide effective management of the damaging local factors (i.e., proteinases and/or bacteria), thereby promoting a local environment supportive of healing. For example, the management of damaging wound exudates, limiting its exposure to the wound bed and surrounding skin, reduces the irritant effects of these exudates, leading to a reduction in the localized stimulation of inflammation. A better understanding of local environment and wound dressing interactions will help in the development of more effective wound dressings in the future.

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HOMOCYSTEINE ASSOCIATED WITH IMPAIRED WOUND HEALING AND DECREASED NITRIC OXIDE PRODUCTION

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Nitric oxide (NO) is a critical mediator of normal tissue repair and is inhibited by homocysteine (Hcy). Following 8 weeks of lower extremity ulcer (LEU) treatment with human fibroblast-derived dermal substitute (Dermagraft®) for 12 ($n = 12$) patients, a correlation was observed between patients with a poor wound healing response to Dermagraft and elevated serum homocysteine (Hcy). The responders group (R) to Dermagraft treatment ($n = 6$) was observed with robust granulation tissue, epidermal migration and a 67% rate of healed ulcerations. All R-group patients (6/6) had normal serum Hcy. The nonresponders (NR) group ($n = 6$) exhibited poor granulation tissue formation and epidermal migration and a complete absence of healed wounds. Five of the NR patients (5/6) were observed with elevated serum Hcy. There was no significant ($p < 0.05$) difference between the wound areas ($cm^2 \pm SE$) of each group [35.43(±28.99)-R vs. 19.90(±7.55)-NR]. After 2 weeks of treatment, the R-group demonstrated significantly greater %Δwound area than the NR-group [62.17 (±7.96)-R group vs. 23.17(±8.82)-NR group; $p < 0.05$]. Wound fluid nitrate (WFNOx), a surrogate measurement ($\mu M \pm SE$) for local NO bioactivity, was significantly lower for the NR-group than the R-group [3.17(±1.46)μM-NR group vs. 12.98(±1.73)μM-R group; $p < 0.05$]. In a large group of LEU patients ($n = 138$) we observed a 50% incidence of elevated Hcy; 69% for diabetic neuropathic LEU patients and 47% for nondiabetic LEU patients. Hcy inhibits NO production by multiple pathways and may also inhibit wound repair by occupying the fibronectin domain of fibrin during provisional matrix formation. Our study suggests that elevated serum Hcy is a common finding in chronic LEU patients. The high Hcy may contribute to impaired wound healing by decreasing wound NO production and, perhaps, by inhibition of fibronectin-fibrin-mediated wound matrix development.

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CHANGES IN THE LEVELS OF ADIPONECTIN AND MCP-1 MAY BE RESPONSIBLE FOR CIGARETTE SMOKE-INDUCED ATHEROSCLEROSIS

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Atherosclerotic plaque formation involves recruitment and adhesion of circulating monocytes to sites of blood vessel wall injury followed by their migration into the subendothelial space stimulated by MCP-1 (monocyte-chemoattractant-protein1). Monocytes differentiate into macrophages, cells that release a variety of factors and take up oxidized LDL, becoming foam cells. Smooth muscle cells of the vessel wall differentiate and migrate to the subendothelial space, interact with foam cells and components of the ECM to form a "fatty streak," the precursor of plaque. Adiponectin, a protein secreted by adipocytes, circulates in the blood, maintains blood vessel wall stability and inhibits foam cell formation. Cigarette smoke is a major risk factor of atherosclerosis, although the mechanisms remain unknown. We showed previously that both firsthand and secondhand cigarette smoke stimulate MCP-1 in endothelial cells and others have shown that adiponectin levels in smokers with cardiovascular disease are reduced. We hypothesized that a localized increase in MCP-1 and a decrease in adiponectin are critical for cigarette smoke-induced plaque formation. To test this possibility, we used mice transgenic for human ApoB100, a model system that closely mimics human conditions that lead to atherosclerosis, and a smoking machine that closely simulates human smoking. We found that, in smoking mice, the micro vessels in the heart tissue are often filled with lipids, the areas surrounding the plaque-prone regions have numerous neutrophils, that these cells express high levels of MCP-1, and that the plaques are larger than in control mice. In addition, the level of the adiponectin monomer in the blood of smoking mice is lower and the presence of this protein in the heart tissue is also decreased. In conclusion, the decrease in adiponectin and the increase in MCP-1 levels may be responsible for cigarette smoke-induced atherogenesis.

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SITE-SPECIFIC ALTERATIONS IN THE EARLY INFLAMMATORY PROCESS OF WOUNDS

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Wound healing is a multistep process that concludes with scar formation. A common clinical observation is that oral wounds exhibit privileged healing compared to extra-oral wounds. Current data demonstrates reduced inflammation and more rapid reepithelialization of oral wounds suggesting that reduced inflammation is vital to privileged repair. To examine the initiation of inflammation, the present study examines IL-1α, IL-β and TNFα production by oral versus cutaneous keratinocytes and compares mast cell infiltration in oral versus cutaneous wounds. Two 1 mm diameter excisional wounds were placed on the lateral tongue or dorsal skin of anesthetized BALB/c mice. At 3, 6, 12, and 24 hours after injury, wound tissue was harvested for analysis. Cytokine expression levels were measured by semiquantitative RT-PCR normalized to actin. At every time point, levels of IL-1α, IL-β and TNFα mRNA were less in oral than dermal wounds. When analyzed by ELISA, IL-1 protein levels peaked at 6 hours in both oral and cutaneous wounds, yet the levels were lower in oral wounds. Mast cell infiltration was measured by counting toluidine blue positive cells in fixed wound sections. The number of mast cells/mm^2 was decreased overall in oral mucosal wounds. These findings suggest that a decreased early inflammatory response to injury may be responsible for the privileged repair of oral mucosa.

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THROMBIN STIMULATES INTERLEUKIN-8 PRODUCTION IN HUMAN MACROPHAGES THROUGH A JNK AND NFκB PATHWAY

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Wound healing is a complex biological event orchestrated by various resident and infiltrating cell types spanning multiple stages. Thrombin, a multifunctional serine protease released upon wounding, stimulates fibroblasts and macrophages at the sites of wounds to express, chemokines, such as interleukin-8 (IL-8). We have used cellular and molecular approaches to investigate the mechanisms involved in thrombin induces IL-8 expression in human macrophages. Western blot analysis and RT-PCR show that thrombin stimulates IL-8 in a rapid and dose-dependent manner. Various inhibitors for signal transduction proteins were used to delineate the pathways involved. We found that, at nanomolar concentrations, inhibitors for both c-Jun N-terminal Kinase (JNK) and NFÉ»B strongly inhibited the thrombin-induced production of IL-8 suggesting that they are major regulators of this stimulation. JNK was shown to be phorsphorylated in a time-dependent manner in response to thrombin treatment confirming its involvement. In order to identify the mediator of JNK and the thrombin receptor, we use affinity pull down and found that Rho GTPase was activated by thrombin suggesting that Rho may be the mediator of JNK activation. In addition, we found that PKC is a negative regulator of thrombin-induced IL-8 expression. Our study broadens the horizon of how IL-8 is regulated in wound healing and may help develop pharmaceutical methods to manipulate this process in pathological conditions.

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THE SELECTIVE ANTI-INFLAMMATORY ACTIVITY OF PROLONGED RELEASE NANOCRYSTALLINE SILVER DRESSING (ACTICOAT 7®) IN THE TREATMENT OF CHRONIC VENOUS LEG ULCERS

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The treatment of venous ulcers must start with compression and if the ankle brachial index is greater than 0.8 high compression bandages can be applied. Despite edema control, there are a number of venous ulcers that do not heal at the expected rate. Patients with venous ulcers of greater than 4 weeks duration were treated with a prolonged release absorptive nanocrystalline silver dressing (Acticoat 7) under the 4 layer bandage, Profore for 12 weeks, or until healing. Biopsies were obtained from the ulcer base at week 0 for histology and bacterial burden. Duplicate biopsies for quantitative bacteriology were performed with one submitted whole and the second bisected into superficial and deep components. The paired biopsies were then repeated after a median of 6.5 weeks (range 2 to 12 weeks). The histological specimens were examined by the histopathologist (SR). Inflammatory infiltrates were identified in the superficial, middle and deep segments of the biopsies. Acute infiltrates were identified through the concentration of neutrophils and chronic infiltrates by the presence of lymphocytes. Each biopsy and each segment was graded for infiltrates on a four point semi quantitative score. A total of 15 patients (9 male, and 6 female) were enrolled into the study. The median age was 63 years (range 30–83 years). The median duration of current ulceration was 17.3 weeks (range 4 weeks to 11 years) and the median ulcer area was 4.8 cm^2 (range 1.8–43.9 cm^2). The median exposure to Acticoat 7 was 82 days (range 8–86 days). There were 12 sets of paired biopsies that were analyzed. There was a statistically significant reduction ($p = 0.0114$) in the $\log_{10}$ (total bacterial count) between the baseline and final biopsies (median 4.48 and 3.00, respectively). Four patients healed, 8 patients continued to the end of the 12-week study period and three patients were discontinued early. For all patients, the median percentage reduction in ulcer area was 94.4% and the median final ulcer area was 0.4 cm^2. Analysis of the histology and bacteriology data demonstrated that the presence of a high neutrophilic infiltrate in skin biopsies was associated with high bacterial counts (superficial compartment of the quantitative biopsies) at week 4 and delayed healing ($p = 0.037$). In the week 0 biopsy, increased lymphocytic infiltrates within the superficial and middle segments were associated with accelerated healing in the first 4 weeks ($p = 0.26$ and 0.09). The nanocrystalline silver dressing has demonstrated an anti-bacterial and permissive but selective anti-inflammatory action allowing lymphocytic infiltrates to increase associated with an accelerated reduction in ulcer size.

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SYSTEMIC EFFECT OF VACUUM ASSISTED CLOSURE® (V.A.C.®) THERAPY IN PORCINE FULL THICKNESS ACUTE WOUNDS

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Vacuum Assisted Closure® (or V.A.C.®)* Therapy is the negative pressure wound therapy provided by the V.A.C.® System, available from KCI USA, Inc. This study was conducted in young Yorkshire swine to evaluate the effect of V.A.C.® locally and systemically on wound healing and cytokine levels in wound fluid and tissue and in serum. Two full thickness, excisional wounds were created on the dorsum of swine. In Group 1 (n = 6), one wound received V.A.C.® Therapy using a polyurethane foam V.A.C.® dressing and 125 mm Hg continuous negative pressure, and the second wound received DuoDERM®** without negative pressure. Group 2 animals (n = 4) served as controls and received only DuoDERM® on both wounds. Blood, wound fluid, and biopsy samples were taken at predetermined intervals and the levels of 10 cytokines and growth factors, the acute-phase inflammatory biomarker C-reactive protein (CRP), and immunoglobulins M & G were tested by ELISA. Preliminary data from the first phase of the study (n = 2 swine/group) indicated a trend toward lower serum CRP values and higher serum IgM and IgG levels in swine treated with V.A.C.® Therapy compared to controls. The remaining swine are being evaluated to determine whether V.A.C.® Therapy moderates the local inflammatory wound healing response. In addition, testing of splenic lymphocyte and peripheral blood lymphocyte, neutrophil, and monocyte functions are being performed to study possible systemic effects on the immune system.

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** DuoDERM® is a trademark of E.R. Squibb & Sons, Inc.

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ALKALINE PHOSPHATASE ACTIVITY IS RELATED TO ACUTE INFLAMMATION AND COLLAGEN TURNOVER DURING ACUTE AND CHRONIC WOUND HEALING

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Alkaline phosphatase (ALP) is an ubiquitous enzyme produced by different cell types. Previous reports have demonstrated ALP induction by extracellular matrix, ascorbic acid or IL-6. Using histochemical techniques, we evaluated the ex vivo activity of ALP in incisional and excisional acute wounds, chronic wounds, and keloids in animal and human models. We also compared in situ ALP activity with type I/III collagen levels using histological methods (Herovici technique). Results demonstrated that 1) during the first stage of acute wound healing the increase in ALP activity precedes high levels of type III collagen, meanwhile type I collagen diminishes. When ALP activity disappears, the proportion of type I/III collagen returns to normal. 2) ALP activity is almost null in chronic wounds, which are characterized by thick type I collagen fibers and some type III collagen throughout the tissue. However, when the wound is treated, ALP activity rises to maximum levels together with type III collagen, but type I collagen is absent. 3) In keloids ALP activity is also absent; however when the scar is treated with a proinflammatory cytokine downmodulator (collagen-polyvinylpyrrolidone) endogenous type I collagen diminishes, while type III collagen and ALP activity are increased. Based on the above results we suggest that ALP activity is an acute inflammation marker since the enzyme levels are increased in acute wounds but not in chronic inflammation processes. Nevertheless, when chronic lesions or scars are in the process of healing, the increase in ALP activity is clear. And as a result, we believe that treatments for chronic wounds or scars induce acute inflammation and consequently stimulate wound repair or fibrolysis. In conclusion, ALP activity is an important factor to be considered in acute and chronic wound repair and their healing process.

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THE INFLUENCE OF INHIBITION OF POSTBURN STRESS ON WOUND SEPSIS IN SEVERELY SCALDED RATS

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Objective: To investigate the effects of hibernation drugs on early wound sepsis in severely scalded rats.
Methods: Sprague-Dawley rats inflicted 30% TBSA deep thickness were randomly divided into A (immediate resuscitation) and B (immediate resuscitation and lytic cocktail). After infusion of 10^8 CFU/ml Pseudomonas aeruginosa in the wound on third postburn day, survival was compared between two groups. Local tissue samples and plasma were collected and the plasma level of IL-2 mRNA, IL-4 mRNA, IL-10 mRNA, and IFN-γ mRNA were assayed by fluorescence PCR.
Results: Compared with Group A, host survival significantly increased (0.663 ± 0.026, $P < 0.01$), viscera injury decrease in group B after infusion bacteria. The plasma Th1 cytokine mRNA (IL-2, IFN-γ) levels in B group were significantly increased compared with A group. However, the plasma Th2 cytokine mRNA (IL-4, IL-10) levels in group B were not as significantly increased as group A.
Conclusions: Immediate resuscitation with hibernation drugs can decrease viscera injury after sepsis, increase host survival and improve host immune system by preventing Th1 cell from over activation and decrease Th2 cytokines.

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AMYGDALA LESIONS INCREASE IL-8 AND MIP-1[ALPHA] GENE EXPRESSION IN DERMAL WOUND TISSUE

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Cortisol has been shown to impact upon wound healing through its immunosuppressive properties, and its release is modulated by the central nucleus of the amygdala (CeA) which is involved in fear and stress reactions. This study assessed the role of the CeA in modulating proinflammatory chemokine expression following wounding, under both stress and nonstress conditions. Bilaterally CeA-lesioned rhesus monkeys and nonoperated controls received separate dermal wounds on 3 consecutive days. Six hours postwounding, tissue biopsies were obtained from each wound. Following this, animals were subjected to 2 h of confinement as a stressor. Thus, examination of tissues on day 1 represented a nonstressed condition, and on days 2–3 a stressed condition. Using real-time PCR, we determined mRNA expression for IL-8 and MIP-1[alpha] in wounded and unwounded tissue, two chemokines critical in the recruitment and activation of neutrophils and monocytes in response to tissue injury. Analyses revealed that tissue mRNA levels for IL-8 and MIP-1[alpha] were increased in both groups following wounding. However, these rises were significantly greater in CeA-lesioned animals than controls. Repeated stress (confinement) attenuated these increases in mRNA expression equally in both groups (days 2–3). These results indicate that the inflammatory response to skin wounds is modulated, in part, by the amygdala. However, stress-induced alterations in inflammation are not CeA dependent for this particular stressor. Importantly, this finding indicates that a lesion of a central brain structure can impact upon peripheral wound healing parameters.

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PROSTAGLANDIN E2 REGULATES NORMAL AND KELOID FIBROBLAST MIGRATION AND COLLAGEN SYNTHESIS: IMPLICATIONS FOR WOUND HEALING

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Background: Prostaglandin E2 (PGE2) is an important inflammatory mediator, which has been shown to regulate fibroblast chemotaxis and extracellular matrix production through four G-coupled protein receptors (EP1, EP2, EP3, EP4). Previous studies have linked abnormal PGE2 signaling with the development of improper damage repair and tissue fibrosis in the airway. This study begins to address the role of PGE2 in dermal fibrosis, particularly keloid formation. Such an analysis is consistent with data indicating inflammatory/immune abnormalities associated with keloids.
Hypothesis: Keloid fibroblasts respond to PGE2 appropriately, but in a quantitatively diminished manner.
Methods: Cultured fibroblasts from normal human skin and keloid lesions were used for these studies. Fibroblast migration was assayed using a well established two dimensional motility assay. Collagen production was analyzed using Western blot and commercial ELISA methodology.
Results: Administration of PGE2 decreased keloid fibroblast migration in a dose-dependent manner, via a mechanism which appears to involve the EP2/EP4-cAMP-PKA signal transduction pathway. PGE2 reversed the transforming growth factor β-1-induced increase in collagen type I in both normal and keloid fibroblasts.
Discussion: Our hypothesis is partially supported by the results, which show that the response of keloid fibroblasts to PGE2 is qualitatively and quantitatively identical to normal fibroblasts. PGE2 decreases the rates of migration and collagen synthesis of keloid fibroblasts, raising the possibility that exogenous PGE2 may have potential therapeutic applications to reduce keloid formation.

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THE ROLE OF SERINE PROTEASES AND THEIR INHIBITORS IN CHRONIC WOUNDS

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The role of proteases in chronic wounds has been the subject of many investigations in recent years. These studies have reported biochemical differences between chronic and acute wound fluids and have shown that elevated levels of proteases, in particular the matrix metalloproteinases are abundant in chronic wounds. While this has led to the hypothesis that the chronic wound environment is hostile and not conducive to wound repair, it is still unknown whether this is due to a direct or indirect defect in protease regulation or their inhibitors.
We hypothesize that an excess of proteases, specifically the serine proteases present in chronic wounds, are primarily responsible for this hostile wound environment. These proteases degrade endogenous growth factors, reducing their efficacy and delaying healing.
In this study, fluid and tissue from chronic and acute wounds were collected over a 24-hour period. Using ELISA, samples were assessed for protease activity (specifically elastase and typsin-like enzymes), their inhibitors and growth factors.
Our results show that serine proteases, predominantly elastase, were significantly elevated in the chronic wound fluids. The serpins designed to control these proteases were not up-regulated when compared to the acute controls, resulting in excessive proteolytic activity. An increase in serine protease production without an increase in their serpins is thought to result in a reduction in growth factors, an effect, which was also observed in the chronic wound samples.
This work indicates that there is an imbalance in the ratio of inhibitor to enzyme, due to an up regulation of protease production in chronic wounds. We also conclude that misregulation of serine proteases may be responsible for the observed excessive degradation of the extracellular matrix and growth factors in these chronic wounds.

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THE EFFECTS OF HYDROGEN PEROXIDE ON FETAL WOUND REPAIR

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Cutaneous wound healing is a complex process that ends with scar formation in adult skin. In contrast, fetal skin within the first and second trimesters of development heals without a scar. One of the most important aspects of scarless fetal wound repair appears to be the lack of inflammation in these wounds, suggesting that acute inflammation can promote scar formation in the skin. While it is well accepted that inflammation causes scar formation in the fetus, it is not known what specific factors produced during inflammation are responsible for these effects. The present experiments tested the possibility that oxidants released by activated inflammatory cells can contribute to scar tissue production by inducing inflammation in the fetus. Using a murine fetal wound repair model we demonstrate that hydrogen peroxide increased E15 (embryonic day 15) fibroblast proliferation and ultimately interfered with scarless healing in E15 skin, possibly through the induction of TGF-β1. Defining the factors produced during the inflammatory response that contribute to scar formation could be important for the development of new therapies designed to minimize adult scarring.

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COVERAGE OF EXPOSED BONE IN PRESSURE ULCERS WITH A FIBROBLAST-POPULATED CONSTRUCT DECREASED HEALING TIME COMPARED TO CONTROLS IN A PILOT RANDOMIZED, CONTROL TRIAL

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Patients who are hospitalized, elderly, or have spinal cord injury develop stage IV pressure ulcers in sacral, ischial, and trochanteric regions. Bone exposure of these pressure ulcers becomes a significant clinical problem. We propose that treatment of exposed bone with bone resection and a fibroblast-populated construct (FPC) (Dermagraft) accelerates healing. Four patients with stage IV sacral or ischial pressure ulcers where randomized to the control group and underwent bone resection with wet-to-dry dressing changes. Four other patients were randomized to the treatment group and underwent bone resection with application of FPC. Both groups were clinically monitored and treated with culture-specific antibiotics. The data showed that patients treated with FPC had their exposed bone covered after an average at 2.25 weeks, while the control groups required at least 4 weeks for bone coverage. Therefore, the preliminary data suggest that FPC is effective in reducing the time of bone exposure after sacrectomy/ischiectomy in pressure ulcer surgery.

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PRESSURE ULCER PREVENTION: AN AGGRESSIVE QUALITY IMPROVEMENT INITIATIVE CENTERED ON A HOSPITAL-WIDE BED REPLACEMENT PROGRAM-

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Advocate Christ hospital is a 650-bed, level-one tertiary-care center that created a comprehensive wound care program in November 1998. Over the first 3 years of the program there were over 140 admissions per year directly from the wound center along with numerous hospital to hospital transfers for complex wound management. Prevalence rates therefore increased linearly over this time frame. The wound team calculated a system-wide (eight hospitals) spend of $2.1 million per year for specialty rental beds. Incidence rates for the system were at, but not lower than, national levels despite the high cost incurred. A $1.3 million capital purchase for KCI atmos-air 9000† mattresses resulted in the complete replacement of all medical surgical beds in all eight hospitals. An internal prevalence and incidence team was created and data were collected both at the hospital and system-wide level on a quarterly basis. Hospital and system incidence rates fell to or below 7% (targeted goal) and the entire project will achieve a return on investment in 18 months. Data to be presented include prevalence and incidence results, rental bed costs pre- and postbed purchase, and a detailed review of the process of creating an electronic prevalence and incidence tracking system.

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LASER DOPPLER PERFUSION IMAGING FOR THE ASSESSMENT OF HEALING IN PRESSURE ULCERS

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Clinically the outcome of pressure ulcers is predicted by the severity/stage of the ulcer, patient health and health care options. These factors, however, cannot be used to accurately evaluate the prognosis and healing in these ulcers, thus delaying any required change in the treatment plan. Techniques are needed that can help in the assessment of healing and improve the clinical management of these ulcers. Studies of tissue health such as angiogenesis, by measuring blood flow in the ulcer at different time points, has been examined as a way to evaluate the state of healing ulcers. Although the Laser Doppler Perfusion Imager (LDPI) has been used to assess various wounds, there is a need to validate it as a predictor of wound healing. The standard clinical outcome parameter for skin wounds has been time to heal. To validate the LDPI as predictor of healing, however, a measure that gives periodic feedback such as healing rate is a more useful outcome measure.
The goal of this study therefore was to determine a protocol for the LDPI and evaluate its ability to predict healing in pressure ulcers. Previous studies have indicated that perfusion values obtained after heat stress may reflect the blood flow more accurately than absolute perfusion values. It was hypothesized that the difference in the blood perfusion after heat provocation is expected to correlate with the healing rate.
The selected heat provocation (increase of 20 °F) was evaluated on five spinal cord injured pressure ulcer patients. The LDPI assessment and healing rate measurements were done weekly for 5 weeks.
Preliminary results suggest a relationship between the difference in the perfusion and the healing rate for the following week. Further studies will be necessary to determine the exact relationship. This tool has the potential to play a critical role in the clinical management of pressure ulcers as well as other skin ulcers.

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FLUID VOLUME MEASURMENTS: THE GOLD STANDARD?

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Introduction: Accurate wound size measurement is vital to assessment of progressive healing. Lack of an accepted "gold standard" is a major obstacle to assessment of any effective wound management regimen.
Methodology: Sixteen pelvic region pressure ulcers were monitored weekly for 3 weeks. All wounds were stage III or IV, equally divided between sacral and ischial locations. Linear wound size was measured using the Decubitus Disposable Measuring Guide (DDMG). Wound size index was defined as the sum of the linear dimensions. Wound volume was determined by filling the covered wound cavity with fluid; the volume of saline required to fill the wound was a direct measure of wound volume.
Results and Discussion: Correlation between the two measurement methods was determined utilizing Spearman's rank correlation coefficient. Wound size index decreased significantly ($p < 0.01$) over the study period. Wound volume changes were nonsignificant. Closer evaluation of assessment methodology found missing wound volume data, due primarily to patient positioning difficulties. All missing data points were for ischial wounds. Wound absorption of measurement fluid and residual fluid in the wound prior to measurement could not be determined. Wound size index may be influenced by subjective evaluation of wound boundaries.
Conclusion: Wound size index significantly discriminated longitudinal changes in wound size, while volume measurements did not. Wound volume measurements have greater measurement errors than wound size index.

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NEUTROPHIL ENZYMES IN PRESSURE ULCERS

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It has been speculated that a prolonged and elevated neutrophilic response has an important role in the pathogenesis of chronic wounds. This has raised the possibility that one or more of these proteases might be useful as a diagnostic or prognostic biomarker for chronic wounds. The purpose of this study was to examine several primarily neutrophil-derived proteases present in pressure ulcer tissues and to determine whether protease activity could be correlated with neutrophil levels. Biopsies of pressure ulcers were taken from consented subjects ($n = 13$) and again after 3 months standard care. Mean wound volume decreased 2.4 cm^3 per month; however, volumes of six wounds were increased at the end of the study. Levels of extractable myeloperoxidase (MPO), elastase, collagenase-2 (MMP-8), and gelatinase (MMP-9) activities were determined. Mean levels of MPO ($n = 11$) at study initiation and after 3 months were nearly identical (2945 and 2845 milliunits per mg extract, respectively). Interestingly, these levels were similar to that previously found in healing open dermal wounds at 4 days postwounding. MPO levels increased in the extracts obtained from three subjects and declined in five subjects. Elastase (corr. 0.265 $p = 0.233$) and MMP-8 (corr. -0.05 $p = 0.835$) activities correlated poorly with MPO. This is likely a reflection of differences in the access of protease inhibitors (e.g., $alpha_1$-antitrypsin, TIMP, and $alpha_2$–macroglobulin) to these wounds. As expected, a greater correlation level was observed between the proteases (MMP-8 vs. elastase corr. 0.362 $p = 0.097$; MMP-8 vs. MMP-9 corr. 0.55 $p = 0.009$). The results from this study highlight the high degree of variability that can be observed in the pressure ulcer environment. This further suggests that a combination of biomarkers will be required for making accurate diagnostic assessments of chronic wounds.

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STEM CELLS POSTERS

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AUTOLOGOUS BONE MARROW-DERIVED CELLS FOR ACCELERATING THE HEALING OF HUMAN CHRONIC WOUNDS

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We have previously shown that resident fibroblasts from human chronic wounds are phenotypically abnormal, displaying an impaired response to TGF-beta 1, decreased TGF-beta Type II receptor expression, and decreased phosphorylation of Smad2, Smad3, and p42/44 MAPK. We now report that these human cells delay healing by approximately 30% when implanted in mouse incisional wounds and in a dorsal flap model. These new findings point to the need for the transplantation of new healthy cells in human chronic wounds. Bioengineered living skin constructs can deliver new cells to chronic wounds, at least for a limited period of time. However, most available bioengineered skin constructs consist of already differentiated allogeneic cells, which do not allow for the possibility of engraftment and reconstitution of wound structures. Recently, in a preliminary report, we have shown that autologous bone marrow-derived cells can dramatically enhance the healing of recalcitrant chronic wounds when all other treatments have failed. Our present efforts are now focused on characterizing the bone marrow cells involved in this stimulation of wound healing and in developing better ways for their topical delivery to the wound. Here we present evidence that a fibrin construct may be an ideal way of applying bone marrow-derived cells to human chronic wounds. Autologous bone marrow cells are allowed to grow in culture under conditions favoring the survival and proliferation of mesenchymal stem cells (MSC), as shown by surface markers and functional studies. This usually requires two weeks after bone marrow harvesting. The cellular component is then placed in a fibrinogen solution which, when simultaneously combined with thrombin through a CO_2 driven flow, delivers a fine cell-containing fibrin polymer film to the wound. In vitro and in vivo experiments, both in mouse models and in human wounds, show the feasibility of this approach.

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SYSTEMIC VS. TOPICAL TRANSPLANTATION OF ENDOTHELIAL PROGENITOR CELLS FOR WOUND HEALING

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Previous wound healing studies have attempted to improve angiogenesis (new blood vessel ingrowth). Recent studies suggest, however, that revascularization of wounds may also be accomplished by vasculogenesis: endothelial progenitor cells (EPCs) from the bone marrow and blood that home to sites of tissue damage and form new blood vessels. The goal of this study was to evaluate the use of systemic and topical transplantation of EPCs for wound healing in an in vivo rabbit model. It was hypothesized that applying cells topically through an EPC-seeded albumin scaffold may promote revascularization from inside the wound versus only from the periphery. Further, it was hypothesized that combining topical and systemic transplantation of EPCs may increase vessel formation from both directions. Full thickness wounds were created on the dorsum of New Zealand White rabbits. Wounds were treated with either an EPC-seeded scaffold, nonseeded scaffold, or no treatment (control). Additionally, half of the animals were given systemic injections of EPCs. Digital pictures were taken weekly until sacrifice to determine healing rates. Laser Doppler Perfusion Imaging scans were used to measure changes in blood perfusion during healing. Animals were sacrificed at 2 or 4 weeks; wounds were excised and prepared for histological examination. Histomorphometry was used to calculate epithelialization and contraction rates, as well as the volume fraction of cell nuclei and blood vessels. Preliminary results suggest that animals that received systemic transplantations of EPCs had higher overall healing rates than those with no injection. Systemic transplantation alone improved healing over the control by the end of 2 weeks. When combined with a topical scaffold (both EPC-seeded and nonseeded), systemically injected cells showed improved healing by the end of the first week.

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PERIPHERAL BLOOD FIBROCYTES IN FIBROPROLIFERATIVE DISORDERS (FPD) OF THE SKIN

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Introduction: Fibrocytes are a unique bone marrow origin leukocyte subpopulation which display fibroblast-like properties and are antigen-presenting cells that are up-regulated systemically after burn injury. This study investigated their role in FPD of the skin using leukocyte-specific protein-1 (LSP1) specific for fibrocytes in HTS, keloids, and mature scar.
Materials and Methods: Fibrocytes were cultured from PBMC isolated from burn patients and normal individuals. Skin biopsies were taken from burn patients with hypertrophic (3) and mature scar (3) and normal skin (20) as well as patients with keloids (3). Double immunostaining (IHC) with antibodies to LSP1 and to the C-propeptide of type I collagen were performed on cryosections to identify fibrocytes in tissue. Extraction of fibrocytes from keloids, HTS, mature scar, and normal skin allowed quantification of fibrocytes by flow cytometry.
Results: LSP1 was found in both fibrocytes and lymphocytes, but not in fibroblasts. Western blot analysis showed that LSP1 expression was significantly higher in fibrocytes than in lymphocytes obtained from all five patients examined. Fibrocytes in hypertrophic scar were seen as duallabeled spindle-shaped cells. In normal skin, fibrocytes were present in the upper layers of the dermis and <1% of cells extracted, whereas, approximately threefold more fibrocytes were found in HTS and primarily in the papillary dermis. Preliminary data suggests that normal dermis contains $0.51\% \pm 0.35$ fibrocytes, mature scar $0.30\% \pm .031$, keloids 1.18 ± 0.22, and HTS 1.12%.
Conclusions: Fibrocytes are up-regulated systemically after injury and in FPD in skin where they appear to be up-regulated contributing to the development of keloids and hypertrophic scarring. More investigation is required to determine the functional roles of these bone marrow derived cells in fibroproliferative disorders.

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FORMULATIONAL DEPENDENCE OF HUMAN MESENCHYMAL STEM CELL BEHAVIOR IN FIBRIN SEALANT

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Objective: The use of fibrin as an injectable cell carrier to repair osseous defects has been reported with varying degrees of success. This study investigates fibrin formulational dependence on human mesenchymal stem cell (hMSC) proliferation and differentiation.
Methods: 2×10^4 hMSCs (Bio Whittaker) per fibrin gel were suspended in fibrin formulations with varied fibrinogen (5, 17, 50 mg/ml) and thrombin (250, 167, and 1 U/ml); and cultured in 24-well plates at 37 °C, 5% CO_2 for up to 2 weeks. Formulational influences on clot structure were evaluated by confocal microscopy and electron microscopy. hMSC proliferation and morphology were monitored by calcein staining. hMSC-conditioned medium was assayed using zymography (MMP-2, MMP-9), fibrin overlay (uPA), and reverse overlay (PAI-1) to examine the effect of fibrin formulation on protease activity and fibrinolysis.
Results/Discussions: Confocal and electron microscopy revealed formulation dependence on three-dimensional fibrin structure, with lower fibrinogen concentrations yielding more open, homogeneous microstructures. Although hMSCs are viable in all fibrin formulations investigated, proliferation rates varied with fibrinogen concentration, with lower fibrinogen concentrations (i.e., 5 mg/ml) promoting greater hMSC proliferation. Protease levels and activities were sensitive to culture time and fibrin formulation. PAI-1 expression was elevated in hMSCs inside fibrin gels, while uPA activity was low. Both the pro and active form of MMP2 was detected by gelatin zymography, but no MMP9 was detected. This study suggests that fibrinogen:thrombin ratio can significantly influence hMSCs proliferation and interactions, and should be carefully considered when developing formulations for optimal delivery of hMSCs.
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MRI MONITORING OF OSTEOGENESIS OF HUMAN BONE MARROW STROMAL CELL-BASED TISSUE ENGINEERING CONSTRUCTS

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Bone marrow stromal cells (MSCs) are a promising cell resource of osteoprogenitor cells for bone tissue engineering. However, the diverse characteristics of osteoprogenitor cells within the bone marrow of individual subjects require varying treatments to stimulate osteogenic differentiation. Thus, an effective monitoring system is needed to identify the progression of osteogenesis. Magnetic resonance (MR) microscopy was used in the present study to monitor osteogenesis of tissue engineering (TE) constructs prepared by human bone MSCs seeded on scaffolds of gelatin sponges. The characteristics of MR images and parameters corresponded to osteogenic progression of TE constructs exposed to differentiation medium, significantly differing from control groups exposed to basic medium. Upon quantification, MR image and parameters correlated well to cell seeding densities and alkaline phosphatase activities of various TE constructs. In conclusion, MR can effectively detect the biochemical cascades of osteogenic differentiation of TE constructs and may be a promising, noninvasive monitoring system to provide three-dimensional information for bone tissue engineering.