Fourteenth Annual Meeting and Exhibition of the Wound Healing Society

Sheraton Atlanta Hotel
Atlanta, Georgia
May 23–26, 2004

Summary Program

Sunday, May 23, 2004

18:00 Keynote Address – Robert Nerem, Ph.D., Georgia Institute of Technology
Blue Ribbon Poster Presentation (Abstracts 1–10)
Opening Ceremony

Monday, May 24, 2004

08:30 Opening Remarks
Announcement – Blue Ribbon Industrial Poster Award Winners

General Session: The Inflammatory Response

09:00 The Macrophage and Coordination of the Inflammatory Response – John Christman, M.D., Vanderbilt University
09:30 Inflammatory Cytokines – Anne Richmond, Ph.D., Vanderbilt University
10:00 Refreshment Break

Breakout Sessions

10:30 Inflammation/Cytokines (Abstracts 11–17)
Clinical Studies (Abstracts 18–24)
Growth Factors (Abstracts 25–31)
12:15 Symposium Lunch

General Session: Young Investigator Awards

14:00 Young Investigator Award Presentations (Abstracts 32–37)
15:30 Refreshment Break
16:00 Young Investigator Award Presentations (Abstracts 38–41)
17:00 Poster Reception: Wine & Cheese

Tuesday, May 25, 2004

07:00 Continental Breakfast

Breakout Sessions

08:30 Gene Therapy (Abstracts 42–48)
Oxygen & Oxidative Stress (Abstracts 49–55)
Wound Infection (Abstracts 56–62)
10:15 Refreshment Break

General Session: Strategies for Tissue Repair

11:00 Regeneration of the Damaged Heart – Piero Anversa, M.D., New York Medical College
11:35 Recruitment of Endothelial Precursor Cells to Sites of Tissue Repair – Timothy Crombleholme, M.D., Cincinnati Children's Hospital and the University of Cincinnati School of Medicine
12:30 Symposium Lunch

Dual Track Plenary: Acute Wounds

14:00 The Effect of the Perioperative Environment on Acute Wound Healing – Andrea Kurz, University of Bern
14:30 Nitric Oxide and Acute Wound Healing – Adrian Barbul, M.D., Sinai Hospital/Johns Hopkins University
15:00 Ventral Hernias – Michael Franz, M.D., FACS, University of Michigan Health System and the VA Ann Arbor Health Care System
15:30 Smoking (Cessation) and Surgical Wound Complication – Lars Tue Sorensen, M.D., University of Copenhagen

Dual Track Plenary: Oxidative Stress/Ischemia

14:00 Role of Reactive Oxgyen Species in Wound Healing – Chandan Sen, Ph.D., The Ohio State University Medical Center
14:30 Stress, Oxygen, Nitric Oxide, and Wound Healing – Phillip Marucha, D.D.S., University of Illinois at Chicago

15:00 HIF-1α and its Downstream Targets – Wayne S. Zundel, Ph.D., University of Colorado Health Sciences Center
15:30 Hypoxic Interactions – Thomas Mustoe, M.D., Northwestern University Medical School
16:00 Refreshment Break

General Session

16:15 3M Award Presentation
Business Meeting

Wednesday, May 26, 2004

07:00 Continental Breakfast

Dual Track Plenary Session: Cell–Matrix Interactions

08:30 Mechanisms of Feedback Regulation by Collagen-Binding Integrins – Thomas Krieg, M.D., University of Cologne
09:00 Integrin-Mediated Regulation of Generators of Angiogenesis – Ambra Pozzi, Ph.D., Vanderbilt University School of Medicine
09:30 A Reevaluation of Integrins as Mediators of Angiogensis – Dwayne G. Stupack, Ph.D., The Scripps Research Institute

Dual Track Plenary: Diabetic Wounds

08:30 Off-Loading – Lawrence Harkless, D.P.M., University of Texas Health Science Center at San Antonio
09:00 Debridement – Christopher Attinger, M.D., Georgetown University
09:30 Control of Diabetic Foot Infections – Peter Sheehan, M.D., Hospital for Joint Diseases
10:00 Refreshment Break

Breakout Sessions

10:30 Biomaterials/Bioengineering (Abstracts 63–69)
Epithelization (Abstracts 70–76)
Stem Cells (Abstracts 77–83)
12:15 Symposium Lunch

General Session: Bioengineering

14:00 Endothelial Responses to Inflammation – Robert A. Swerlick, M.D., Emory University School of Medicine
14:30 Biomaterials for Vascular Repair – Elliott L. Chaikof, M.D., Ph.D., Emory University School of Medicine
15:00 Refreshment Break

General Session: Engineering of the Repair Process

15:15 Tissue Engineering of Bone – Barbara Boyan, Ph.D., Georgia Institute of Technology
15:45 Neural Tissue Engineering –Michelle Laplaca, Georgia Institute of Technology
16:15 Signaling at the Cell–Materials Interface – Andres Garcia, Georgia Institute of Technology
16:45 Neurite Extension on Biomaterials – Ravi Bellakonda, Georgia Institute of Technology

Program subject to change

All abstracts for presentation at the Annual Meeting are included in the following compilation. Abstracts are numbered and categorized according to the meeting schedule.

Abstracts No.	**Session Name**
1–10	Blue Ribbon Industrial Posters*
11–17	Inflammation/Cytokines
18–24	Clinical Studies
25–31	Growth Factors
32–41	Young Investigator Awards
42–48	Gene Therapy
49–55	Oxygen & Oxidative Stress
56–62	Wound Infection
63–69	Biomaterials/Bioengineering
70–76	Epithelization
77–83	Stem Cells
84–153	Posters

* Blue Ribbon Industrial Posters 2, 3, 4, and 5 will also be presented in podium sessions as presentations 76, 26, 28, and 81, respectively.

BLUE RIBBON INDUSTRIAL POSTER SESSION

001

A NOVEL MODEL FOR THE ASSESSMENT OF FORMULATED ENZYMATIC DEBRIDING AGENTS

Lei Shi, Justin Keen, Braham Shroot
DPT Laboratories, San Antonio, TX USA

In order to closely mimic the actual mechanism of wound debridement by an enzymatic debriding agent, a synthetic wound eschar substrate (SWES) has been developed. The SWES is prepared by the enzymatic conversion of fibrinogen to fibrin in the presence of fibrous bovine collagen and elastin, to form an insoluble planar matrix composed of the three major proteins present in wound eschar. Each of the proteinaceous components is uniquely labeled prior to formation of SWES with different dye, and the debridement test is conducted using a Franz Diffusion System. The debriding agent is applied to the SWES mounted on the donor compartment of the cell, and the digested hydrolysate containing dye-labeled peptide fragments is sampled from the receptor compartment. Accordingly, the progress of digestion is obtained in real time. Since the three proteins are labeled with spectrally distinct dyes, data on the hydrolysis of the substrates can be collected simultaneously. This method facilitates the testing of formulated debriding products in a manner that closely mimics application to a wound and provides a powerful tool for the evaluation of formulated enzymatic debriding agents.

002

AUSTRALIAN TAIPAN SNAKE VENOM TO SYNTHETIC OXYNOR FOR WOUND HEALING

Binie V. Lipps,
Ophidia Products, Inc., 11320 South Post Oak #203, Houston, Texas 77035

At Ophidia Products, Inc. we have discovered Oxynor a synthetic therapeutic from snake venom for wound healing. The venom of Australian taipan snake (*Oxyuranus s. scutellatus*) is extremely potent due to the presence of taipoxin. The intact complex molecule of taipoxin having molecular weight 45.6 kDa is composed of three subunits α, β and γ. Fractionation of crude venom by High pressure chromatography (HPLC) using ion exchange column permits separation of the subunits. The mitogenic activity was revealed in non toxic β taipoxin. Non toxic β taipoxin showed mitogenic activity on variety of eukaryotic cells. Its activity as a mitogen extended to wound healing in experimental animals. We identified the active domain for wound healing in β taipoxin. after trypsin digestion were separated on HPLC, resolved into 11 different fragments. Each fragment was tested on PC12 cells to test mitogenic/neurotrophic activity. The fragment which showed the highest activity consisted of 21 amino acids. A synthetic peptide consisting of ten amino acids, from the N-terminal — was named **Oxynor**.

Oxynor mimics the biological properties of natural β taipoxin when tested in vitro and in vivo systems. The biological results showed that Oxynor was not toxic at the concentration of 100 μg/ml for PC12 cells and 500 μg/adult mouse. Oxynor was mitogenic to various eukaryotic cells. Oxynor is neurotrophic showing neurite outgrowth on PC12 cells. Growth of human skin fibroblast cells indicates that Oxynor has keratinous activity. Experimentally produced 4 mm punched wounds on the back of mice were treated with β taipoxin 100 μg/mouse or Oxynor at 500 μg/mouse for seven consecutive days. The results showed complete closure of the wounds after six days while the wounds of control treated with PBS were open. Histology examination of the skin around the wounds revealed that treated mice showed re-epithelization of the epidermis, which looked close to the normal mouse skin biopsy. Whereas the controls showed distortion of epithelium. β taipoxin and Oxynor were tested for in vivo at the concentration of 100 μg/ml in hydrogel vehicle applied once daily to help heal 6 mm ischemic skin wounds in rats. At days 7 and 14 wound treated with β taipoxin were about 15% smaller than the wounds treated with Oxynor. This shows that synthetic Oxynor is as efficacious as natural β taipoxin under the conditions of the test. On increasing the concentration of Oxynor to 500 μg/ml should give equivalent wound closure to 100 μg/ml of β taipoxin. In 1997 the US FDA approved for sale the first recombinant human platelet-derived growth factor (rhPDGF) for treatment of human non-healing wounds. Oxynor will be next therapeutic for wound healing Currently, Oxynor is in clinical trials, it has passed toxicity tests.

003

α1-ANTICHYMOTRYPSIN – A HUMAN SERPIN WITH THE POTENTIAL TO HEAL DIABETIC ULCERS

A. Goppelt[1], W. Hans[1], Y. Shi[2], M. Bittner[1], J.-P. Halle[1], P. Hof[1], J.M. Davidson[2,3].
[1]SWITCH Biotech AG, Floriansbogen 2–4, D-82061 Neuried, Germany [2]Department of Pathology, Vanderbilt University, [3] VA Medical Center, Nashville, TN 37212, USA

α1-Antichymotrypsin (α1-ACT) is a secreted serine proteinase inhibitor of the serpin family. It is strongly upregulated during the inflammatory phase of wound repair to control the undesirable destructive side effects of cathepsin G which is released from infiltrating neutrophils. We have shown that α1-ACT and its mouse homologue spi2 exhibit a classic acute phase response after cutaneous injury in murine and human skin. The induction of spi2 gene expression following wounding is significantly less pronounced in diabetic mice. Transient overexpression of spi2 or α1-ACT significantly increased wound tensile strength in two independent diabetic models: in genetically diabetic mice, gene gun mediated delivery of spi2 or α1-ACT cDNA increased the average wound strength by 42% ($P = 0.001$) and 21% ($P = 0.013$) at d5 post-wounding respectively. In a STZ induced diabetic rat model the breaking strength of adenovirally spi2- or α1-ACT-infected wounds increased by 20% ($P = 0.049$) and 23% ($P = 0.004$) at d7 after injection respectively. Moreover, the topical application of human α1-ACT protein to wounds of diabetic mice had a significant and dose dependent effect on tensile strength at d5 post-wounding (21% increase, $P = 0.003$). Histological analyses of these wounds indicated less infiltration of neutrophils in the treated wounds. α1-ACT appears to be a key mediator between proteolysis and cytokine agonism and antagonism. Our data indicate that this balance is disturbed in diabetic wounds and that α1-ACT might act as a switch to initiate wound healing in diabetic ulcers.

(Supported by SWITCH Biotech AG)

004

NOVEL PLATELET CONCENTRATE PREPARATION SYSTEM FOR GROWTH FACTOR MEDIATED WOUND HEALING

Sukavaneshvar S[1], Zheng Y[1], Mohammad SF[1]
[1] Hemogenesis, Salt Lake City, UT, USA.

A promising approach for advanced wound healing involves the use of growth factors from autologous platelet concentrate (PC). A novel PC (NPC) preparation technique, based on platelet aggregation and filtration, has been developed to concentrate platelets (in less than 5 minutes) in a fully disposable system, eliminating the need for a centrifuge or cell saver.
NPC was compared with centrifugation-based PC (CPC) with regard to: 1) platelet recovery, 2) growth factor content, 3) cell proliferative activity with monkey epithelial cells and bone-marrow mesenchymal cells (BMMC), 4) healing response of Porcine full-thickness wounds. There was no statistically significant difference in platelet count, growth factor content (platelet-derived growth factor enzyme-linked immunosorbent assay), and cell proliferation activity (Fig. 1) between NPC and CPC. Quantitative assays (Alkaline phosphatase and 5-bromo-2-deoxyuridine) in BMMCs also showed that NPC was equal to CPC in its mitogenic activity ($n = 6$, paired t-test).
The histopathology of porcine full-thickness wounds (at 24 days) showed that NPC and CPC (in a hydrogel carrier) treated wounds healed similarly. The NPC and CPC treatments resulted in less scarring, less inflammation, and improved vascularity than hydrogel alone.
The NPC's growth promoting ability is equivalent to CPC, and the technique is significantly simpler, faster, and more economical than CPC. Thus, NPC could dramatically expand the use of PC for wound healing when combined with various wound dressings and adjuvants.

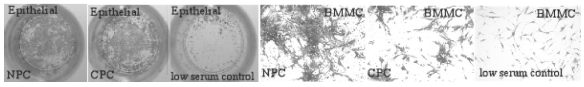

Fig. 1. Images showing similar cell growth with NPC and CPC.

Acknowledgements: This study was funded by a grant from the NIH.

005

CYCLODEXTRIN POLYMER BASED BIOCOMPATIBLE MATRIX FOR LOCAL GENE DELIVERY

D.W. Kang[1], N.C. Bellocq[2], T. Schluep[1], L.D. Looper[1], D-L. Gu[1], E.M. Quijano[1], S.F. Wen[1], X. Wang[2], G.S. Jensen[2], S.H. Pun[2], M.L. Zepeda[1], M.E. Davis[3]
[1]Canji Inc., San Diego, CA USA; [2]Insert Therapeutics Inc., Pasadena, CA USA; [3]Chemical Engineering, Caltech, Pasadena, CA USA

Biocompatible matrices, such as bovine collagen, have demonstrated usefulness in delivering gene therapy vectors that express growth factors to local environments for tissue repair. Unlike animal derived matrices, we have developed a new synthetic matrix consisting of a linear cyclodextrin-polyethyleneglycol co-polymer that is non-covalently cross-linked with di-adamantane-polyethyleneglycol via inclusion complex formation between adamantane and cyclodextrin (CD-Ada). We performed both *in vitro* and *in vivo* experiments for biocompatibility and localized transgene expression using a recombinant adenovirus (rAd) vector containing either the reporter gene, GFP, or the therapeutic gene, PDGF-B. *In vitro* results demonstrated cell migration, adenoviral transduction, and gene expression with no visible signs of toxicity in human skin fibroblasts. Qualitative gene expression from the CD-Ada containing rAd was delayed by approximately two days when compared to collagen, but the level of expression was greater over a longer period of time. *In vivo* experiments demonstrated gene expression after local delivery to mouse skin using rAd-GFP in CD-Ada. Again, the expression was slightly delayed but duration of expression was comparable to collagen. Expression studies using rAd-PDGF-B, were performed in the rat polyvinyl alcohol sponge model and showed comparable quantitative DNA and RNA levels between CD-Ada and collagen (DNA: 4.1×10^{10} and 4.5×10^{10} MEQ of PDGF-B/assayed sponge, respectively; RNA: 7.0×10^{8} and 3.2×10^{8} MEQ of PDGF-B/assayed sponge, respectively). Additionally, we explored the use of plasmid DNA with the CD-Ada matrix and observed PDGF-B expression *in vivo*. Our results show that this new delivery system provides a safe, efficient, and adaptable medium for both viral and non-viral gene delivery.

006

DEVELOPMENT OF A NOVEL MATRIX METALLOPROTEINASE-INHIBITING WOUND DRESSING

Gary A. Skarja, Maud B. Gorbet, Rebecca K. Lawson-Smith, Michael H. May, Michael V. Sefton*
Rimon Therapeutics Ltd., Toronto, Ontario, Canada, *Institute of Biomaterials and Biomedical Engineering, University of Toronto, Ontario, Canada

Rimon Therapeutics develops synthetic therapeutic polymers (Theramers™) that combine bioactivity with a broad range of desirable mechanical and physical properties. The MI Theramer™ is a polymer that modulates the activity of matrix metalloproteinases (MMPs).. Excessive proteolytic cleavage of extracellular matrix and growth factors resulting from elevated levels of MMPs is believed to contribute to the impaired wound healing associated with chronic, non-healing wounds. MI Theramer™ beads suspended in ThermaGel™ (a thermoreversible gel) make up MI-Gel™ Dressing. The dressing may be applied as a liquid that is poured into a wound, where it will gel on contact. The dressing can be reliquified and removed easily without re-injuring the wound by cooling it.
In vitro inhibition of collagenase type IV (equivalent to MMP-2) from clostridium histolyticum (Molecular Probes) was measured. A dose-dependent inhibition of MMP-2 activity was measured for MI Theramer™ beads alone and suspended in ThermaGel™ indicating that MMP-2 is able to diffuse into the gel and bind to the beads suspended therein. *In vivo* efficacy was demonstrated by observing the degradation of glutaraldehyde cross-linked gelatin tubes using a mouse subcutaneous pouch model. Gelatin zymography on extracts collected from the gelatin tubes co-implanted with MI Theramer™ beads also showed reduced MMP-2 and MMP-9 activity in comparison to controls.
The insoluble MI Theramer™ beads were able to modulate MMP activity both *in vitro* and *in vivo*. The beads retained their inhibitory activity when they were suspended in a novel thermoreversible gel (ThermaGel™). The combined MI-Gel™ Dressing may have promise as a novel chronic wound healing product.

We would like to thank Ping Xu for his assistance in the collection of the gelatin implantation study data.

007

REBALANCING WOUND BIOCHEMISTRY IMPROVES HEALING: A CLINICAL STUDY EXAMINING EFFECT OF PROMOGRAN

B. Cullen, L. Kemp, A. Essler, K. Wallenfang-Sohle, R. Stadler
Johnson & Johnson Wound Management, Gargrave, N. Yorkshire, UK
Dept. Dermatology, Minden Hospital, Germany

Normal wound healing is a carefully controlled balance of new tissue formation and destructive processes necessary to remove damaged tissue. Within this complex environment there are many points of regulation, which control the biological processes necessary to achieve wound repair. An alteration in any of these processes can result in an imbalance of the biochemical components, which ultimately results in delayed wound closure and the formation of a chronic wound. Therefore, we propose that an effective therapeutic approach would modify this hostile environment and redress this imbalance.
In this study we have evaluated the effect of PROMOGRAN *in vivo*, in patients with venous leg ulcers. Wound fluid samples were collected and analyzed from a number of patients prior to and during treatment with PROMOGRAN.
Our results have shown that wounds, which respond to PROMOGRAN treatment, have also shown a decrease in protease activity in the corresponding wound fluid samples. Whilst it is impossible to determine whether this reduction in protease levels is responsible for healing or merely symptomatic of other changes occurring within the wound, we have shown that a decrease in proteolytic activity is concomitant with healing.
This study provides clinical evidence that PROMOGRAN can rebalance the chronic wound environment *in situ* and thereby promotes wound repair.

Type acknowledgements here.

008

CROSS – TALK BETWEEN FIBROBLASTS AND KERATINOCYTES IN 3-D FIBRIN CLOTS. IN VITRO STUDIES

N. Sese, M. Cole, and B. Tawil
Baxter BioScience, BioSurgery, Westlake Village, CA USA

Fibrin Sealant products such as Tisseel[R] have been used over the last 25 years to reach hemostasis during surgery or to seal tissues. Moreover, there were many studies on the use of fibrin sealant in cell and bioactive substance delivery. Our In Vitro and In Vivo studies, over the last 2 years, have focused on the use of fibrin sealant to deliver human fibroblasts or keratinocytes to overcome the healing deficiency in chronic wounds. We have shown that some fibrin formulations supported a high fibroblast proliferation and other fibrin formulations supported a high proliferation of human keratinocytes. In this study, we examined the use of fibrin sealant in the co-delivery of both human – derived keratinocytes and fibroblasts. The study report examined the cell proliferation of these two cell types in various formulations of fibrin sealant. Fibroblasts and keratinocytes were mixed with various dilutions of the Sealer Protein solution and added to culture plates before adding the Thrombin solution to form fibrin clots containing both cell types. We found that a low to medium sealer protein concentration (1–34 mG/mL) and a very low thrombin concentration (1 U/mL) in the final fibrin clots provided for an optimal cell proliferation for both cell types within these fibrin clots. This profile of proliferation was different from that seen when keratinocytes alone or fibroblasts alone were incorporated in the fibrin clots. Morphologically, it was difficult to determine the cell type from examining cell morphology, thus, it was difficult to determine the cell distribution. In conclusion, we found that various modified formulations of fibrin sealant may be chosen when co- delivering fibroblasts and keratinocytes. Moreover, there seems to be a positive feedback between keratinocytes and fibroblasts when they were co – introduced in the fibrin clots. This feedback could be carried by growth factors. Future studies will determine his signaling process.

009

EVALUATION OF AN OZONE-BASED WOUND MANAGEMENT SYSTEM

A.J. Denvir[1], A.C. Blankenburg[1], I.A. Holder[2]
[1]Lynntech Inc., College Station, TX, USA [2]Shriners Hospital for Children, Cincinnati, OH, USA

The onset of infection can lead rapidly to sepsis, septic shock, and eventually death. Considering the high costs of hospitalization and the added trauma and discomfort to the patient, improved methods for infection control are needed. Ozone offers a specific solution to the problem of effective management of microbial wound contamination. Despite the advantages, several technical barriers have prevented ozone-based disinfection treatments from becoming more widely investigated. Negative press from years of unsubstantiated medical successes has made the medical community wary of even legitimate ozone technologies. Because there has been no research into using ozone in infection prevention, much of the hardware has not been developed to the stage where the technology can be used in clinical trials. Lynntech Inc., over the past 5 years, has been working to develop a pre-clinical ozone-based wound management system. This new hardware uses an electrochemical ozone generator that can deliver a predetermined quantity of ozone gas to a specific wound site. Electrochemically generated ozone has been shown to be effective against a range of gram negative and gram positive bacteria and our studies with various mimetic wound systems have shown that with controlled delivery, ozone can be utilized as either a disinfectant or as a biostat. The device is well suited for hospital use as it operates of low voltage power supplies and unlike other ozone generation technologies will not interfere with other electronic equipment while it is in operation. This paper outlines both the advantages and limitations of using an ozone based system as a wound management tool and looks to the future research that needs to be performed to substantiated the initial research findings.

This work was funded through NIH

010

STRUCTURAL CHARACTERIZATION OF SMALL INTESTINAL SUBMUCOSA (SIS) USING CRYO AND TRADITIONAL SCANNING ELECTRON MICROSCOPY (SEM)

A.D. Janis[1], D.M. Sherman[2]
[1]Cook Biotech Incorporated, West Lafayette IN [2]Life Science Microscopy Facility, Purdue University, West Lafayette IN

Composition and structure determine the fate of biomaterials in the body, encouraging or discouraging acceptance, cellular infiltration, and degradation or incorporation into host tissue. Most biomaterials research has been focused on composition, while structure remains a relatively uninvestigated factor in biomaterial success. Naturally derived biomaterials may provide optimal structure for cellular repopulation in certain applications. Porcine small intestinal submucosa (SIS) is an acellular, naturally occurring extracellular matrix used in the clinical treatment of soft tissue injury, including pressure, venous, and diabetic ulcers. Numerous studies have demonstrated that SIS induces host tissue infiltration, tissue-specific remodeling, and repair. SIS consists primarily of collagen, although the presence of other proteins, growth factors, glycosaminoglycans, and proteoglycans has been reported. SEM was used to characterize the cellular environment provided by this biomaterial. Cryo-SEM was used to image mechanically isolated, oxidatively disinfected, lyophilized, sterilized, and rehydrated SIS. The fractured cross-sectional faces of intact intestine, oxidatively disinfected SIS, and lyophilized SIS were imaged with traditional SEM. Comparison of the images utilizing cryo and traditional preparation techniques show that SIS is a heterogeneous matrix consisting of textured fibers of mixed size. There are differences in the structure of the mucosal and serosal surfaces. Although the matrix architecture shows signs of collapse and delamination following lyophilization, much of the native structure is preserved. These changes are preserved following rehydration. The effects of these structural changes on cellular reaction and infiltration are unknown, but the naturally complex architecture-clearly facilitates host interaction on thecellular and subcellular levels. This work was supported by Cook Biotech Incorporated.

INFLAMMATION/CYTOKINES

011

INFLAMMATION-INDUCED ANGIOGENESIS: POTENTIAL ROLES OF IL-8. AND VEGF

M. Yao, M. Dueck, and M. Martins-Green
University of California, Riverside, CA USA

Inflammation is invariably accompanied by angiogenesis; both are important in wound healing. However, it is not known how angiogenic factors that are present during the inflammatory phase of wound healing are involved in inflammation-induced angiogenesis. In this study, we address the contribution of two such factors, interleukin-8 (IL-8/CXCL8) and VEGF, in inflammation-induced angiogenesis. We determined the relative levels of these molecules during the first two weeks of the wound healing process in partial-thickness skin wounds of rabbits. IL-8 levels increase rapidly after wounding, reaching maximal levels after 24 hr; in contrast, VEGF levels peak at 3 days. We also determined the time course of the infiltration of various inflammatory cells and production of new blood vessels after wounding. Neutrophils were markedly increased 4 hr after wounding, and their level peaked at 24 hr. Monocyte infiltration peaked 48 hr after wounding and decreased by day 3. The number of blood vessels was significantly increased by day 3 after wounding and continued to increase through days 5 and 7.
In order to determine the contribution of IL-8 and VEGF to the angiogenic process, we performed the wounding experiments in the presence and absence of IL-8 and VEGF antibodies. Treatment with IL-8 antibodies blocked the early stage of blood vessel formation but did not alter the late stage; in contrast, VEGF antibodies blocked the late stage without altering the early stage. Currently, we are performing studies using inhibitors against IL-8 and VEGF receptors to determine whether we observe similar results; we are also beginning to decipher molecular mechanisms involved in this process. This study suggests that IL-8 and VEGF play complementary roles in stimulating angiogenesis during wound healing.

This work was funded in part by AHA and TRDRP

012

MOLECULAR MECHANISMS OF THROMBIN-INDUCED INTERLEUKIN-8 EXPRESSION IN HUMAN MACROPHAGES

L. Zheng; M. Martins-Green;
Cell Biology and Neuroscience Department; University of California, Riverside, CA

Wound healing involves a series of overlapping, highly coordinated events. Macrophages play multiple crucial roles in these processes including the secretion of chemokines. Interleukin-8 (IL-8) is an inducible chemokine that is expressed in a variety of cell types present at the wound site, including macrophages, and is known to contribute to chemotaxis, angiogenesis, and tissue remodeling during wound healing. One of the potent natural inducers of IL-8 is thrombin, a multifunctional enzyme that is released upon wounding. Little is known about how this enzyme stimulates IL-8 in macrophages. Therefore, we used a variety of cellular and molecular approaches to investigate the signal transduction mechanism of thrombin-induced IL-8 expression in THP-1-differentiated macrophages. We show that stimulation of IL-8 by thrombin occurs in a rapid manner that is time- and dose-dependent and can be abolished by addition of hirudin, a specific inhibitor of thrombin. At the mRNA level, IL-8 peaks $\sim$4 hrs after treatment and declines by 9 hrs. A variety of inhibitors were used to delineate the signal transduction pathways leading to IL-8 expression. Application of AG1478, a potent EGFR inhibitor, abolishes thrombin-induced IL-8 production suggesting EGFR transactivation. We further show that MEK1/2 and p38 are important signal mediators; their inhibition abolishes IL-8 production. Contrarily, PKC inhibitors do not abolish, but rather enhance production of IL-8, whereas activators inhibit the thrombin stimulation, suggesting that PKC is a negative regulator of IL-8 expression. In summary, these results suggest that thrombin stimulates IL-8 expression by transactivating the EGFR leading to activation of MEK1/2 and p38, and that PKC is a negative regulator of this stimulation process.

Supported by AHA and TRDRP.

013

VARIABLE P53 RESPONSE IN A 3D MATRIX

M.A. Carlson[1] and M.T. Longaker
[1]University of Nebraska Medical Center, Omaha, NE; [2]Stanford University, Stanford, CA

Detachment of an attached (stressed) fibroblast-populated collagen matrix (FPCM) induces fibroblast apoptosis; this may be secondary to increased p53 after detachment. We have noted, however, that the p53 response after detachment of an attached FPCM (i.e., stress-release) varies among cells strains. We hypothesized that modulation of p53 after FPCM stress-release may be related to the fibroblast population density at the time of release. Human foreskin fibroblasts (initially seeded at 0.5×10^6/ml in 0.2 ml) were cultured for 48 hr in attached collagen matrices prior to detachment for 0–6 hr; p53 levels and lysate DNA concentration (used as a measure of fibroblast population density) were determined with immunoblot densitometry and a spectrophotometer, respectively. The experiment was performed with 15 different strains of fibroblasts. The p53 level increased (defined as $\geq$100% increase in baseline p53 level) during the 6 hr detachment period in 6/15 (40%) of fibroblast strains (responders), and downregulated or unchanged in the remaining strains (nonresponders). The mean DNA concentration in the responders *vs.* nonresponders was 1.56 μg/ml (median 1.63, range 0.89–2.25) *vs.* 2.57 (median 1.94, range 1.38–5.09), respectively ($p < 0.05$, Wilcoxon rank sum test). Stress-release of the FPCM resulted in a variable p53 response which appeared to have a relationship with the fibroblast population density; a lower density was associated with p53 upregulation after detachment. This variable p53 response may in turn be related to varying proliferative capacity in the collagen matrix, as there was a broad range (nearly an order of magnitude) of DNA concentration among the 15 cell strains after 48 hr of attached FPCM cuture.

Supported by a grant from the NIH (K08 GM00703).

014

MACROPHAGE SUSPENSIONS TREATMENT OF INFECTED STERNAL WOUNDS FOLLOWING CABG SURGERY

David Danon[1], Adi Zuloff-Shani[1], Erez Kachel[2], Raphael Mohr[4], Eilat Shinar[1] and Arie Orenstein[3]
[1]Research and Development unit, M.D. A national Blood Services, [2]Department of Cardiac Surgery, [3]Department of Plastic Surgery, Sheba Medical Center, [4]Thoracic and Cardiovascular Surgery, Sourasky Medical Center, ISRAEL

Macrophages serve as the coordinators of the wound healing process. Since 1998 following the Israeli Ministry of health authorization, macrophage suspensions have been used for the treatment of ulcers, in more than 800 elderly and paraplegic patients suffering from decubital chronic ulcers.
As previously published, a significant number of genes showed increased levels of expression in hypo-osmotic shock activated cells, using DNA microarrays technique. The majority of these genes are considered to be directly involved in the macrophage function and in the wound healing process.
Macrophge suspensions are prepared from a whole blood unit of healthy, young volunteer blood donors in a closed, sterile system, as previously described. The activated cells are applied to the wounds either by local injection or by direct deposition to the wound.
Between January 2000 and October 2003, 112 patients with postoperative sternal wound infection were treated with macrophage suspension. Full closure of the wounds was achieved in 104 (93%) of the patients.

Original wound surface area	8–175 cm2 (mean 93)
Days until treatment	6–180 (mean 47)
Days until 50% closure	6–60 (mean 21)
Days until full closure	10–138 (mean 49)

No side effects were noted.
The use of macrophage suspension is a safe and effective therapeutic strategy that reduces risk of complications and morbidity and improves the quality of life for long -suffering patients. Length and cost of hospital stay may be reduced, as the treatment requires no hospitalization.

015

IMPACT OF HYDRATION ON MMP-ACTIVITY

Andrea A. Tandara, Thomas A. Mustoe, Jon E. Mogford
Wound Healing Research Laboratory, Division of Plastic and Reconstructive Surgery, Northwestern University, Chicago, IL 60611

Hydration of keratinocytes modifies the levels of cytokines they secrete, which in turn impacts the secretory behaviour of dermal fibroblasts. In an in vitro coculture model, conditioned media (CM) collagen content was decreased 44% when keratinocytes were hydrated. We hypothesized that this is partly due to increased MMP-activity. We used the same coculture model to study changes in MMP-activity and TIMP secreted by keratinocytes as well as by fibroblasts in monoculture and in coculture in relation to air-treatment or hydration of keratinocytes. Stratified human epidermal keratinocytes (HEK) and confluent human dermal fibroblasts (HDF) were cocultured for 72 h under serum-free conditions. HEK were either kept at the air-interface or hydrated. CM was assayed for MMP-1, -2, -9, TIMP-1 and –2 were assayed using zymograms, western blotting, and ELISA. MMP-1, secreted by both cell types, increased significantly in cocultures compared to monocultures (4-fold in the air-treated group, 26-fold in the hydrated group). MMP-2, secreted mainly by HDFs, was significantly increased by coculture (hydration: 2.4-fold, air: 2.8-fold). MMP-9, predominantly secreted by air-treated HEKs and was significantly decreased in hydrated monoculture (76%) and coculture. HEK-monoculture hydration also significant decreased MMP-1 (86%) and MMP-2 (81%) activity. HDF-secreted TIMP-1 expression was significantly increased by coculture and was unaffected by hydration. Our findings demonstrate that paracrine interactions between HEK and HDF modify MMP activity and that HEK hydration significantly effects on MMP activity. The findings provide insight into the role of hydration on HEK and HDF ctivity during the wound healing process.

016

TNF-ALPHA MEDIATED INDUCTION OF MMP-9 IS MODULATED BY P21-ACTIVATED KINASE (PAK)

Yuan-Ping Han, Ling Zhou and Warren L. Garner
Department of Surgery
The Keck School of Medicine, University of Southern California, CA 90033

Matrix metalloproteinase-9 (MMP-9) transiently expresses in acute wound. In non-healed wounds, MMP-9 together with other proteinases persistently elevate, which may lead excessive ECM degradation and failure of wound closure. To understand the molecular regulation of MMP-9 we investigated the signal transduction for TNF-alpha mediated induction of MMP-9 by dermal fibroblasts. TNF-alpha initiates three major signal pathways including NF-11B, JUN N-terminal kinase (JNK), and p38 MAPK. On the other hand, Rho-GTPase plays an important role in a variety of cellular functions including cell morphogenesis, motility, survival, angiogenesis, and mitosis. It remains unknown if the "cross talk" of these signals having a role in regulation of matrix metalloproteinases (MMPs). In this study we found that over expression of the p21-activated kinase (PAK) specifically attenuates TNF-alpha mediated induction of MMP-9. However, TNF-alpha mediated induction of MMP-3 and proMMP-2 activation was intact. NF-κB signal is regarded as a common pathway for many MMPs. Indeed, PAK did not affect TNF-alpha mediated degradation of Ikappa B, suggesting additional signal is targeted by PAK. In contrast, MMP-3 but not MMP-9 expression is specifically blocked by p38 MAK. Thus TNF-alpha induced expression of multiple MMPs in wound healing may utilize different intracellular signal pathways.

017

LOWER EXPRESSION OF P-SELECTIN MAY MODULATE THE FETAL INFLAMMATORY RESPONSE:

X. Zhu[1], D. Cass[1,2], C.W. Smith[2], O.O. Olutoye[1,2]
Baylor College of Med., Depts. of Surgery[1] & Peds[2], Houston, Texas.

Fetal dermal wound healing is characterized by a minimal inflammatory response and absence of scar. In order to determine the factors contributing to the minimal inflammatory response in the fetus, the role of P-selectin in the interaction and transmigration of leukocytes through vascular endothelium was investigated.
Methods: Primary endothelial monolayers were established from adult porcine central veins and from umbilical veins of mid-gestation porcine fetuses. The ability of the endothelial cells to capture and enable transmigration of adult leukocytes was studied under flow conditions of 4 dynes/sec, with and without prior stimulation of the endothelial cells with TNF-α or IL-1β. In addition, P-selectin mRNA expression was determined by quantitative real time PCR. All data were analyzed by ANOVA.
Results: In response to IL-1β 10 ng/ml stimulation, adult endothelial cells manifested a 10 fold increase in P-selectin mRNA expression while fetal endothelial cells mounted only a 3.5 fold increase. 100 ng/ml was required to mount a 10 fold increase in fetal P-selectin expression while no further increase in adult P-selectin expression was noted at this dose. Leukocyte capture, demonstrated by rolling of neutrophils over the endothelial surface, was more efficient on adult monolayers compared to the fetus. This is inversely related to the rolling velocity which was significantly slower in the adult (5.3 ± 0.6 vs $12.4 \pm 1.0\,\mu m/mm^2$). Ultimately, the number of neutrophils transmigrating across adult endothelial monolayers under flow conditions was significantly higher compared to the fetal monolayer (199 ± 18 vs 72 ± 9 cells/mm^2).
Conclusion: Lower P-selectin expression on fetal endothelial cells may account, in part, for the minimal inflammatory response noted following fetal dermal injury.

Support: RWJF- MMFDP #43485 and Curtis Hankamer Fund (OOO)

019

EFFECTIVENESS OF OASIS® WOUND MATRIX VERSUS REGRANEX® IN TREATING DIABETIC WOUNDS

J. A. Niezgoda
St. Luke's Medical Center, Milwaukee, WI USA

Background: Current care for chronic diabetic wounds involves maintaining wound hydration, providing protection from infection through regular dressing changes, and providing appropriate pressure relief. Even with the best care available, such therapies lead to effective wound healing in only 24% of ulcers within 12 weeks. Treatment of chronic ulcers with recombinant growth factors only moderately improves 12-week healing rates, to 34%. A new, bioactive material, the Oasis® Wound Matrix, comprises a full complement of structural extracellular matrix components and associated growth factors and may be more effective in managing chronic wounds than other current alternatives.
Method: Interim data from 88 evaluable patients in this multicenter, prospective, randomized clinical trial are presented. Patients diagnosed with chronic, full-thickness, diabetic ulcers were enrolled and randomly assigned to receive either weekly treatments of Oasis Wound Matrix or daily treatments of Regranex® becaplermin gel. All wounds were carefully cleaned and irrigated weekly for the duration of the study, with debridement performed as needed; wound areas were recorded to track the rate of wound closure. Incidence of complete wound healing by 12 weeks was evaluated.
Results: Study groups were balanced with respect to patient age and gender. Currently, 53% (24/45) of patients receiving Oasis wound matrix are considered healed versus 35% (15/43) of patients receiving treatment with Regranex.
Conclusion: These data clearly demonstrate Oasis wound matrix is at least as effective as becaplermin gel in managing chronic, full-thickness, diabetic ulcers.

This trial was supported by Cook Biotech Incorporated.

CLINICAL STUDIES

018

HEALING RATE FOR DIABETIC NEUROPATHIC FOOT ULCER

Margolis DJ, Allen-Taylor L, Hoffstad O, Berlin, JA
Department of Biostatistics and Epidemiology, University of Pennsylvania School of Medicine

The goal of this study was to benchmark by year the likelihood that an individual with a diabetic neuropathic foot ulcer (DNFU) will heal. We observed these rates over a more than 10 year period (1988–2000). For this study, we designed a cohort study within the multicenter Curative Health System wound care network of diabetics who have DNFU and whether they healed by the 20th week of care stratified by calendar year.
In total, we evaluated 27, 193 individuals with a DNFU. For, example, between 1988 and 1990 approximately 66% of patients did not heal. By 1999 this percentage had decreased to 49%. Most of the change in improvement occurred before 1998. We noted that the change in the rate of failure to heal was very closely associated with an increase over time in the proportion of patients seen with wounds identified as prognostically favorable as determined by a previously published prognostic model (i.e., individuals with wounds ≤ 2 cm2, wound less ≤ 2 months old, and wound of grade less ≤ 2). Among those most likely to heal, the likelihood of failing to heal went from 62% in 1990 to 32% in 2000. Therefore, the overall decrease in likelihood that a patient with a DNFU would not heal is not just due to the enrollment of less severe cases. There was, however, no real improvement in the likelihood that those with wounds that were unlikely to heal over time and fewer patients with these wounds sought care over time.
In conclusion, those with a DFFU seeking care are more likely to heal today than 10 years ago. This improvement is related to the fact that individuals are seeking care when their wounds are most easily treated and these wounds are now more likely to heal. Unfortunately, those with the most severe wounds are not anymore likely to heal today than they were 10 years ago.

This work was supported by grants DK59154 and AR02212 from the National Institutes of Health

020

VENOUS LEG WOUNDS MANAGED WITH COMPLEX EXTRACELLULAR MATRIX

Eliot N. Mostow, MD, MPH
Akron General Medical Center, Akron, OH USA

Although collagen wound dressings have been used clinically for many years, wound matrix materials comprising the full complement of extracellular matrix molecules have not. Oasis® Wound Matrix (Oasis), a complex, growth factor-containing matrix derived from the intestinal submucosa of pigs, has shown promise as a treatment to manage full-thickness, hard to heal wounds. A multi-center, prospective, randomized clinical trial was conducted to compare the effectiveness of Oasis to a standard of care (SOC) regimen consisting of compression therapy, weekly dressing changes and debridement (as needed) for the treatment of venous leg ulcers. Incidence of healing within 12 weeks, as defined by full epithelialization without drainage, was the primary outcome measure. Significance was determined by the proportion of ulcers healed using Fisher's Exact Test. This interim data included 84 evaluable patients (45 Oasis, 39 SOC). Incidence of healing at 12 weeks was 71% in the Oasis group versus 46% in the SOC group. Fisher's Exact Test yields a significant p-value of 0.018. These results clearly demonstrate that the healing of these hard-to-heal wounds is markedly improved when Oasis wound matrix is used as part of the wound treatment program as compared to standard of care alone.

This trial was supported by Cook Biotech Incorporated.

021

CONTACT SENSITIVITY IN PATIENTS WITH LEG ULCERATIONS: A NORTH AMERICAN STUDY

L. Saap[1], S. Fahim[2], E. Arsenault, [1], M. Pratt[2], T. Pierscianowski[2], V. Falanga[1]×[3], A. Pedvis-Leftick[1]
[1]Roger Williams Medical Center, Providence, RI USA; [2]University of Ottawa, Ontario, Canada; [3]Boston University, Boston, MA USA

Background: Over the last two decades, there have been a number of studies in Europe on contact sensitivity in patients with chronic leg ulcerations with a frequency of positive patch test results ranging from 40 to 82.5%. The prevalence of sensitization has not been studied in North America. Furthermore, many of the newer dressings and wound care products in the market have not been studied for contact sensitivity in patients with chronic wounds.
Objectives: 1) To determine the prevalence of allergen sensitivity in patients with history of leg ulcers in two North American study centers, 2) to compare our results to the European studies and to the North American Contact Dermatitis Group (NACDG) database and 3) to help delineate a standard battery of allergens for patch testing in North American leg ulcer patients
Methods: 54 patients with an active or past leg ulcer were prospectively entered in the study. The patients were patch tested to both the NACDG Standard series, as well as, a comprehensive supplemental series of 48 allergens including wound care medicaments and dressings.
Results: 63% of patients were sensitized to at last one allergen. The most common allergens were Balsam of Peru (29.6%), bacitracin (24.1%), fragrance mix (20.4%), wood tar mix (20.4%), propylene glycol (13.5%), neomycin sulfate (13%), benzalkonium chloride (13%), carba mix (11.1%), nickel sulfate (11.1%) and Duoderm CGF (11.1%). Duoderm CGF was the most allergenic dressing in our study group.
Conclusion: There is a high incidence of positive patch tests in patients with past or current leg ulcerations. Using a modified leg ulcer series along with the standard NACDG series is very important in evaluating patients with leg ulcers.

022

WARMING SURGICAL WOUNDS: EFFECTS ON HEALING AND WOUND COMPLICATIONS

J.D. Whitney, E. P. Dellinger, M. Wickline
University of Washington. Seattle, WA, USA

Aims: Compare postoperative local warming to standard incision care on: 1) healing response and 2) incidence of wound complications.
Methods: This two group randomized pilot study (N = 54) tested the wound healing effects of post-operative warming in patients having gastric bypass or colectomy procedures. Patients randomized to warming received their first 1-hour treatment in the post anesthesia recovery unit followed by 5 additional treatments in the first 48 hours after surgery. Healing response was evaluated by hydroxyproline and mRNA for pro α 1(I) collagen content of a subcutaneous ePTFE implant removed on postoperative day 9. Patients were followed for the first two postoperative clinic visits (6–8 weeks). CDC Surgical Site Infection Criteria were used to evaluate wound infection and complications.
Results: Six warmed (22%) and 10 standard care (37%) patients experienced healing problems.
Incidence of Wound Infection by CDC classification.

Group	Superficial	Deep Incisional	Organ Space
Warming	2	3	1
Standard Care	4	4	2

Mean hydroxyproline levels did not differ, though there was a trend toward higher amounts in warmed patients (332 ± 185 ng/cm ePTFE vs. 286 ± 165 ng/cm). Differences in mRNA for pro α 1(I) collagen approached significance (Chi-Square 5.4, p = .06) with warmed patients showing more cells with abundant positive message.
Conclusions. Clinical and cellular outcomes suggest local incision warming may benefit healing by reducing infection and influencing fibroblast collagen production.

Funded in part through a Fellowship from Augustine Medical.

023

THE USE OF CILOSTAZOL IN THE TREATMENT OF RAYNAULDS DISEASE WITH DIGITAL ISCHEMIA AND TISSUE LOSS

T. Serena, J. Probst
Gannon University; Penn North Centers for Advanced Wound Care 552 Quaker Hill Rd. Warren, PA 16365 USA

Background: More than three quarters of patients with a connective tissue disorder will develop secondary Raynauld's disease. This more severe form of the Raynauld's can lead to digital ischemia, tissue loss and may even result in the need for amputation of one or more digits. Cilostazol is a phosphodiesterase inhibitor currently approved for use in vascular disease of the lower extremities.
Case History: In August of 2002 a 62 year old female pianist with scleroderma presented to the wound clinic with a two year history of intractable digital pain secondary to ischemic ulcerations of the index, long and ring fingers of the right hand. She suffered from secondary Raynauld's disease. Prior unsuccessful treatments included warming the hands, calcium channel blockade, anticoagulation, topical xylocaine and narcotic analgesics. In August of 2002 cilostazol was started at 100mg BID. Wound care consisted of serial debridement, moist wound healing and topical rhPDGF.
Results: Four weeks into treatment the patient's narcotic requirement decreased substantially and the fingers had a pink appearance. The ring finger healed after eight weeks. At three months the patient discontinued narcotic pain medication. Nine months into treatment the index finger had healed and she resumed playing the piano. The long finger finally healed one year after she initially presented to the wound clinic.
Conclusion: Cilostazol may be an important adjunct in the treatment of secondary Raynauld's disease particularly when digital ischemia has developed.
Acknowledgements: Funding from Otsuka Pharmaceuticals.

024

EFFECTS OF AN ELECTRICAL STIMULATION BANDAGE ON WOUND HEALING

J.A. Jennings[1], D.S. Feldman[1], and R.V. Andino[2]
[1]University of Alabama at Birmingham, Birmingham, AL, USA [2]Biofisica, LLC, Atlanta, GA, USA

Although electrical stimulation (ES) has been successful in the treatment of various types of wounds, the popularity of the treatment has waned due to a lack of optimal methods of administration. The goal of this study has been to develop and evaluate a method of delivering ES that is easy to use and suitable for delivery in a home-health environment.[0] Specifically, a novel bandage system developed to provide an electric field in a manner resembling the natural wound current was tested in vivo. In this study, a full-thickness wound model in New Zealand white rabbits was used to measure the effects of such a bandage system on healing of [0]skin defects. Different levels of current were evaluated initially, with 50 and 20 μA selected for the focus of this study, along with a non-stimulated control. Using histomorphometry healing rates, cellularity, and blood vessels were quantified at one and two week time points.
The study showed the ability of this ES bandage to speed the healing process. An increase in overall healing rate over non-stimulated wounds of 45% ($p = 0.04$) was observed in wounds stimulated with 50 μA of current for 1 week. In wounds treated for 2 weeks, contraction rate decreased as well as the ratio of contraction rate to epithelialization rate. The stimulated wounds also showed an increase in the amount of macrophages, with a 79% increase in number of macrophages in wounds stimulated with 20 μA for 2 weeks and a 198% increase in macrophages in those stimulated with 50 μA compared to non-stimulated wounds. The performance of the bandage in the animal model has led to a limited clinical study on pressure ulcer patients using the 50 μA system.
Acknowledgements: This project was funded through Biofisica and the CDC through NCIPC.

GROWTH FACTORS

025

MODULATION OF WOUND REPAIR IN THE OBESE DIABETIC MOUSE: A ROLE FOR VEGF GENE TRANSFER

SB Schwartz, B McCampbell, R Garone, Z Vodslon, E Tokarcsik, J Coico, L Staiano-Coico

Identifying molecular loci of impaired cutaneous healing in diabetes with an eye towards developing targeted therapy to ameliorate dysrepair continues to evolve as a promising area of study. By using an excisional wound model produced on the dorsum of female diabetic C57BL/KsJ *db+/db+* mice as well as their normal (WT) & heterozygous (HZ) littermates, we studied the effects of peri-wound intradermal injection of adeno-associated viral vector (AAV) expressing the 165-amino acid isoform of human vascular endothelial growth factor (VEGF) on the following: kinetics of re-epithelialization, neoangiogenesis and granulation tissue formation, matrix remodelling, collagen deposition, and maturation. One sq. in. full thickness excisional wound was created in the mid-upper back, rendering half of the wound as either right or left paravertebral. Animals were randomized to receive 1 of 3 treatments via intradermal injection: 1)VEGF (AAV) vector; 2)Adnull vector; 3)PBS. Postoperatively, wounds were examined & photographed on Days 3, 7, 10, 14, 21 & 28. Also, tissue was harvested for histology & immunohistochemistry (PECAM), and snap frozen for protein & RNA analysis. A scoring system was used to grade re-epithelialization, granulation tissue thickness, matrix density, inflammation, vascular density, epithelial maturity. AAV-VEGF exerted minimal effect on repair in WT and HZ mice. However, pronounced neovascularization, thickened granulation tissue & increased matrix deposition was noted after VEGF treatment in the db/db mice compared to those that received PBS or adnull vector at all timepoints. While the induction of angiogenesis in VEGF treated db/db mice lagged behind the unimpaired mice by 5–7 days, a global improvement in wound healng was observed.

R Crystal, Dir Inst Genetic Medicine, Weill Med College-Cornell Univ

027

FIBRIN SEALANT COMBINED WITH FIBROBLASTS AND PDGF ENHANCE WOUND HEALING IN EXCISIONAL WOUNDS

J. E. Mogford[1], B. Tawil[2] and T. A. Mustoe[1]
[1]Feinberg School of Medicine, Northwestern University, Chicago, IL USA [2]Baxter BioScience, BioSurgery Westlake Village, CA USA

A number of strategies have been explored for the treatment of cutaneous defects such as protein growth factor or gene therapy approaches. Alternative approaches include dermal or dermal-epidermal skin substitutes or clot substitutes such as fibrin sealants (FS's). Here we test the fibrinogen-thrombin formulation of fibrin sealant combined with fibroblasts and PDGF in wound healing models. Four formulations varying in fibrinogen and thrombin concentration were applied to full-thickness biopsy wounds in the rabbit ear cutaneous wound-healing model with or without cultured rabbit dermal fibroblasts (RDFs; 3×10^5 cells/wound) embedded in the fibrinogen component. At post-wounding day 7, there was no difference in the diluted vs. non-diluted formulations for either the promotion of granulation tissue coverage of the open wounds or total granulation tissue area when tested without embedded cells. Including the RDFs, the highest degree of wound coverage by granulation tissue was observed in the combined dilution formulation (17.3 mg/ml fibrinogen, 167 U/ml thrombin; $n=10$) that was 167% ($p<0.05$) of the non-diluted FS containing cells (50 mg/ml fibrinogen, 250 U/ml thrombin; $n=10$). Inclusion of fibroblasts increased granulation tissue area within the wounds vs. FS alone ($p<0.05$) for each diluted formulation although no differences in this parameter was observed within each group (FS alone or with embedded cells). However, addition of the vulnerary growth factor PDGF-B (3 μg; $n=4$) with the embedded RDFs in the combined dilution formulation increased granulation tissue area over 2-fold ($p<0.01$). Additionally, the presence of the RDFs promoted incorporation of the granulation tissue with and epithelial migration over the FS suggesting an active interaction between cells delivered to the wound by FS and the host repair cells. The findings suggest the progress of cutaneous defect repair can be enhanced by *ex vivo* cell delivery in Fibrin Sealant that is adjusted to balance hemostasis with promotion of wound healing processes.

029

DIFFERENTIAL ANGIOGENIC RESPONSES IN ORAL MUCOSAL AND CUTANEOUS WOUNDS

A. M. Szpaderska, C. G. Walsh, M. J. Steinberg, L. A. DiPietro
Loyola University Medical Center, Maywood, IL

Wound healing in adults is a multi-step process that concludes with scar formation. A common clinical observation is that intra-oral wounds demonstrate privileged healing when compared to wounds at extra-oral sites. Oral mucosa heals faster and with less scar formation than skin does. We have shown that oral mucosal wounds exhibit decreased inflammation and faster reepithelialization than skin wounds. The present study compares angiogenesis in oral mucosal and skin wounds, and examines *in vitro* VEGF production by oral and epidermal keratinocytes. Two 1 mm diameter excisional full-thickness wounds were placed on lateral sides of the tongue and on the dorsal skin of anesthetized BALB/C mice. At 0, 2, 4, 12, 24, 36 hours and 2, 3, 5, and 7 days after injury, wound tissue was harvested for analysis. VEGF and FGF-2 levels were measured by ELISA, and normalized for protein content. The level of VEGF in oral mucosal wounds was less than that of cutaneous wounds at all time points. At 24 hours, VEGF levels peaked in oral wounds, yet were much lower than comparable skin wounds (106.6 +/- 19 vs. 194.0 +/- 23.6 pg/mg protein, $p<0.001$). At 36 hours, wound VEGF levels had declined in oral mucosa, while reaching peak levels in skin (82.7 +/- 8.9 vs. 374.2 +/- 27 pg/mg protein, $p<0.001$). No differences in FGF-2 levels were seen. The relative increase in tissue vascularization during wound repair, analyzed by CD 31 immunostaining, was lower in oral mucosal wounds than in skin (3.4 +/- 0.2% vs. 11.5 +/- 0.5%, $p<0.001$). For *in vitro* experiments, normal human epidermal and oral mucosal keratinocytes were grown for 12 h under hpoxic conditions. In response to hypoxia, VEGF protein production, determined by ELISA, increased 2.3-fold in oral keratinocytes vs. 3.3-fold in epidermal keratinocytes. RT-PCR analysis revealed that relative VEGF mRNA expression increased 2-fold in oral vs. 4-fold in epidermal keratinocytes. This study demonstrates that the differential repair of skin and oral mucosal wounds involves distinct patterns of angiogenesis, and that keratinocytes exhibit intrinsic site-specific differences in VEGF production.

Supported by NIH GM-55238 (LAD) and NIH T32AI07508(AMS).

030

A RABBIT EAR EXCESSIVE SCARRING MODEL USING GROWTH FACTOR STIMULATORS

D-L. Gu, D.W. Kang, D. Looper, D. Maneval, M. Zepeda
Department of Pharmacology, Canji, Inc., San Diego, CA 92121

Research on abnormal scarring, such as excessive scars, has been hampered by the lack of relevant animal models. Recent studies suggest that TGF-β1, PDGF-BB and FGF-2 are stimulators of fibrosis or scarring. We developed a rabbit ear cutaneous excessive scarring model using the growth factor stimulators TGF- β1 (0.25, 0.5 or 1 μg per wound), PDGF-BB (1, 2 or 3 μg per wound) and FGF-2 (0.5, 1, 3 or 5 μg per wound). Three 8-mm diameter wounds were created on each ear in NZW rabbits ($n=6$ wounds). The test growth factors were injected intradermally immediately after wounding. Controls consisted of PBS, BSA and wounding only without injections. Scar thickness of wounds was measured with a micrometer at days 14 and continued twice a week thereafter until sacrifice at day 28. Results showed that the greatest measurement of elevated scars was observed at day 14. Significant differences of scar thickness were observed in TGF- β1 at a dose of 0.25 μg, PDGF-BB at a dose of 3 μg, and FGF-2 at a dose of 3 μg per wound groups when compared to PBS and wounding only groups ($p<0.05$). Interestingly, BSA, a protein stabilizer for TGF- β1 and FGF-2 proteins, also significantly elevated scar thickness at day 14 over PBS alone ($p<0.05$). Trichrome stained tissue sections on day 28 showed an increase in cellularity and thicker epithelium in all doses of TGF- β1, PDGF-BB and FGF-2 protein treated groups when compared to wounding only, PBS or BSA treatments. In general, FGF-2 treated wounds showed more vascularity than TGF- β1 and PDGF-BB treated wounds. By contrast, PDGF-BB treated wounds showed richer collagen deposition than TGF- β1 and FGF-2 treated wounds. Results from these studies demonstrated that TGF- β1, PDGF-BB and FGF-2 contributed to the scar formation in the rabbit ear scar model. BSA may contribute in stimulating scar formation in the early stage of wound healing in this model. Enhancing elevated scar formation in the rabbit ear model will be useful in evaluating anti-scarring agents for excessive scars such as hypertrophic scars and keloids.

031

IGF-I STIMULATES VEGF PRODUCTION IN ENDOTHELIAL CELLS BY INHIBITING POLY(ADP-RIBOSE)-POLYMERASE

S. Beckert, H. Scheuenstuhl, F. Farrahi, R. Aslam, TK. Hunt, Z. Hussain.
Department of Surgery, University of California, San Francisco

Introduction: Vascular Endothelial Growth Factor (VEGF) mediated angiogenesis plays a key role in wound healing. Insulin-like Growth Factor I (IGF-I) has been reported to be angiogenic. However, the mechanism is not known. Recently, a link between transcriptional activity and inhibition of poly(ADP-Ribose)polymerase (PARP) has been reported. We investigated whether IGF-I increases VEGF expression and whether this effect is regulated by the inhibition of PARP.
Material and methods: Subconfluent monolayers of human umbilical vein cells were cultured and serum starved. Cultures were treated with Long-R^3-IGF-I for 20 h. VEGF in the supernatant was measured by ELISA and lactate by a lactate analyser. PARP activity was assessed by measuring the incorporation of ^{14}C-radiolabeled NAD^+. All experiments were performed in triplicate. Results are given as percent change compared to control ± SD; $p < 0.05$ calculated by Student's t-test was considered significant.
Results: IGF-I increased both VEGF (20 ± 10%, 50 ± 16% ($p = 0.01$) and 79 ± 13% ($p = 0.0003$)) and lactate production (12 ± 11%, 20 ± 12% and 48 ± 16% ($p = 0.01$)) in a dose dependent manner (25, 50 and 100 ng/ml). Blocking glucose utilization by 2-desoxyglucose, decreased lactate by 70 ± 11% ($p = 0.0001$), but not VEGF expression. Inhibitors of MAP-kinase (PD 98059) and Proteinkinase C (Staurosporine) reduced the IGF-I effect on VEGF expression by 40 ± 6% ($p = 0.0003$) and 30 ± 7% ($p = 0.01$). 3-Aminobenzamide and nicotinamide alone, inhibitors of PARP, stimulated VEGF production by 66 ± 5% ($p = 0.0003$) and 32 ± 8% ($p = 0.002$), respectively. IGF-I inhibited PARP by 44 ± 3% ($p = 0.01$).
Conclusion: IGF-I enhances VEGF protein expression in endothelial cells. This is mediated through signal transduction and by inhibition of PARP.

Supported by NIGMS grant GM 27345

033

REGULATION OF TGF-β RESPONSES BY A NOVEL ACCESSORY TGF-β RECEPTOR IN HUMAN KERATINOCYTES

A. Marcoux, K. Finnson and A. Philip
Division of Plastic Surgery, Department of Surgery, McGill University, Montreal, Quebec, Canada
Make sure to *underline* the presenting author's name

The potential of TGF-β isoforms to regulate wound healing and scarring is well documented in animal models. Since TGF-β action is likely to be regulated at the level of its cell surface receptors, we analyzed TGF-β receptor profiles and regulation of TGF-β signaling in human keratinocytes. We identified a novel cell surface TGF-β1 binding protein of 150 kDa (r150) on human keratinocytes that interacts with the TGF-β signaling receptors. Further characterization of r150 demonstrated that it is a GPI-anchored protein, that it can be released from the cell surface by an endogenous phospholipase C, and that the released form can bind to TGF-β1. Recent cloning of r150 revealed it to be a novel protein of 1428 amino acids. Our objective was to determine the functional significance of r150 in regulating TGF-β responses in keratinocytes.
Affinity labeling of keratinocytes overexpressing r150 cDNA and immunoprecipitation studies using anti-r150 antibodies show that the cloned cDNA represents r150. Importantly, over expression of r150 results in inhibition of TGF-β1-induced Smad 2 and Smad 3 phosphorylation, gene transcriptional activity, and keratinocyte migration. In contrast, loss of r150 function using antisense morpholino oligos of r150 leads to enhanced Smad 2 and Smad 3 phosphorylation, gene transcriptional activity and proliferation of keratinocytes. In summary, our results demonstrate that r150 is a potent inhibitor of TGF-β signaling in keratinocytes, and that it may have potential therapeutic value in modulating TGF-β action in human diseases where TGF-β plays a pathophysiological role. As such, r150 may be of use in reducing hypertrophic scarring, and an r150 antagonist may promote wound healing.

This work was supported by a 'Canadian Institutes of Health Research' grant and a 'Fonds de la Recherche en Sante du Quebec' award to AP

YOUNG INVESTIGATOR AWARD

032

TRANSCRIPTIONAL PROFILING OF WOUND HEALING PROGRESSION

A. Murad[1] and M.R. Sierra-Honigmann[1]
[1]Cedars-Sinai Medical Center and University of Southern California Keck School of Medicine, Los Angeles, CA

The complexity of cellular and molecular events that occur during wound healing is given by the rapid and continuous changes of matrix structure, cell population, local cytokine composition, nutrient and oxygen availabilities and overall metabolic activity. Angiogenesis is an essential event in the repair of injured tissue. Transcriptional events leading to wound neovascularization begin immediately after the injury has occurred and continue throughout the process until scar remodeling becomes quiescent. This study investigated progression of wound angiogenesis by quantitatively measuring transcripts of multiple genes that participate in wound angiogenesis. The studies were done using a murine model of bilateral 3 mm excision wounds. Wound tissues were collected from 6 hours up to 10 days post-wounding. Each time point consisted of 8 wounds pooled from four different mice. After RNA purification, the samples were analyzed by quantitative real-time RT-PCR using specific TaqMan probe/primer sets for selected transcripts that included the following groups: a) angiogenic factors; b) anti-angiogenic factors; c) receptors; d) cytokines and enzymes; e) matrix proteins; f) signaling molecules and transcription factors; g) apoptosis and proliferation markers; and h) housekeeping genes. A total of 58 genes were profiled including four housekeeping genes. The transcripts revealed several patterns of expression. Some genes such as angiopoietin 1(Ang1), e-selecting, placental growth factor (PlGF), MMP-9 and thrombospondin 1(TSP1) had a clear biphasic pattern where early peaks were detected within the first 24 hours after wounding and were followed by dowregulation and a second increase in transcription after several days. Other transcripts appeared early and had a sustained expression and they include fibroblast growth factor two (FGF2), FGF7, hypoxia inducile factor alpha (HIF-1a) and STAT3. Examples of late transcripts included HOXD3 and alpha smooth muscle actin. Results derived from this study largely confirm previous studies profiling a small number of individual genes such as VEGF, TSP1 and 2 and others. However, the present study integrates complete transcriptional profiles of large number of genes known to have a critical role in healing progression with a particular emphasis on revascularization. A more detailed model of event sequences that lead to tissue reperfusion after injury is derived from the results.

Funded by a Cedars-Sinai grant from the Skirball-Kenis Center for Plastic and Reconstructive Surgery and RO1GM066292 to MRSH.

034

LIGHT-EMITTING DIODE THERAPY INCREASES VEGF AND NO PRODUCTION IN RAW 264.7 CELLS

M. Leong, J. Zhao, L.J. Gould
The University of Texas Medical Branch, Galveston, TX USA

Introduction: Low-energy laser photostimuation has been reported to affect the proliferative phase of wound healing and stimulate macrophages. Light-emitting diodes (LED) are an efficient, inexpensive source of low energy photons. We propose that LED therapy will stimulate macrophage production of VEGF and NO.
Methods: Serum-starved RAW 264.7 cells (5×10^5cells/ml) were treated with or without LPS (10 ng/ml) and IFN-gamma (100 Units/ml) for 24 hours. Cells were divided into groups: 1) control – no LED, 2) 670 nm, 3) 730 nm, 4) 880 nm, and 5) combination (880 nm/730 nm/670 nm) at 4 J/cm^2 per wavelength. Twenty-four hours post-LED, conditioned media was analyzed for VEGF (ELISA) and nitrites (Greiss assay). Proliferation was assessed using BrDU incorporation. Data were analyzed using one-way ANOVA with Tukey post-test.
Results: In serum-starved cells, proliferation was unchanged by LED. VEGF and nitrite levels were increased in groups 4 (VEGF = 1057.1 +/- 100.3 pg/ml; nitrites = 11.13 +/- 0.79 uM) and 5 (VEGF = 1148.9 +/- 88.5 pg/ml; nitrites = 10.72 +/- 1.03 uM) compared to control (VEGF = 812.2 +/- 94.8 pg/ml; nitrites = 7.32 +/- 1.92 uM). LPS/IFN decreased proliferation 25 percent at all wavelengths compared to control + LPS/IFN ($p < 0.01$). Nitrite and VEGF levels were markedly elevated in all five LPS/IFN-treated groups with no appreciable intergroup differences.
Conclusions: LPS/IFN stimulation resulted in maximally elevated levels of nitrites and VEGF, possibly masking the effects of LED. Serum-starved RAW 264.7 cells responded to LED treatment at 880 nm and combined wavelengths with increased VEGF and NO production. These results suggest that LED treatment at 880 nm and 880 nm/730 nm/670 nm may upregulate the production of pro-angiogenic factors by macrophages in the wound bed.

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LEPTIN PLAYS A KEY ROLE IN THE MODULATION OF WOUND ANGIOGENESIS

A.K. Nath[1], A. Murad[2], S.T. Cha[2], and M.R. Sierra-Honigmann[2]
[1]Yale University School of Medicine, New Haven, CT [2]Cedars-Sinai Medical Center and University of Southern California Keck School of Medicine, Los Angeles, CA

Leptin is a pleiotropic cytokine constitutively expressed in adipose tissue and upregulated by hypoxia in sites of tissue injury and in the placenta. Leptin has been demonstrated to play a role in normal and pathological wound healing and it is gene is acutely expressed in experimental wounds. Angiogenesis is among the most salient and well-documented biological actions of leptin. Therefore it was hypothesized that leptin may play in the modulation of wound neovascularization. Using a murine model of experimental wounds the modulation of wound angiogenesis was investigated by treatment of the wounds to either recombinant leptin or a neutralizing anti-leptin antibody. The parameters measured were: a) *wound blood flow* using laser Doppler imaging; b) *vessel density* by counting the number of CD-31 positive vessels in wound sections; c) *angiogenic transcriptional profiling* using quantitative real-time RT-PCR; and d) *validation of transcriptional profiling* by immunohistochemistry and immunoblotting. The treatment of wounds with leptin had a marked effect in wound blood flow that was most significant at 24hours post-wounding with average increase of 55% with respect to the controls. The effect diminished significantly by 72 hours (24%) and became negligible thereafter. Leptin also accelerate wound neovascularization by 24 hours where the number of small caliber vessels averaged 30/mm^2 in leptin treated vessels and 13/mm^2 in the controls ($n = 10$; $P < 0.005$). However, the number of vessels measured by 72 hours not significantly different between controls and leptin-treated wounds. In addition transcriptional profiling reveled changes in gene expression of molecules related to angiogenesis such as VEGF, PlGF, Tie-1, Tie-2, Flt-1, Flk-1, FGF-2 and endothelial markers like CD-31, endoglin and eNOS. Overall, time course analysis throughout the healing process showed that leptin treatment induced an early shift in the expression of many of the analyzed transcripts. The most salient changes could be observed as early as 6 hours post-wounding and included: TSP-1 (+8.7); FLK-1(+9); Ang-2 (+18); CD31(+5.3); endoglin (+7.6); eNOS (+4.2); FGF-2 (+4.3); etc. Leptin mRNA was also significantly increased (+10) as well as the long-form of its receptor ObR-b (+9). Conversely, when wounds were exposed to anti-leptin antibodies, healing progression was impaired and changes in the transcriptional profile showed diminished expression of several genes related to wound neovascularization. Some examples by 24 hours: Endoglin(-2.5); Flk-1(-2.8); eNOS (-5.1); Ang-2 (-5.1); CD31(-3); VEGF-A (-3.6). In conclusion, leptin had and early and significant effect in modulating wound neovascularization. The effect of treatment of wounds with exogenous leptin did not elicit hypervascularization of the wound and appeared to only accelerate what appeared to be a self-limited process of wound angiogenesis.

Funded by a Cedars-Sinai grant from the Skirball-Kenis Center for Plastic and Reconstructive Surgery.

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OVEREXPRESSION OF SMAD7 IN KERATINOCYTES ACCELERATES CUTANEOUS WOUND HEALING

G. Han, A. G. Li, P. Owens, X-J Wang
Oregon Health & Science University, Portland, OR USA

Transforming growth factor β (TGFβ) has both positive and negative effects on cutaneous wound healing. Smad7 acts as a major downstream antagonist of TGFβ signaling in keratinocytes and its role in wound healing has not been defined. We have established a Smad7 transgenic mouse line using a keratin 5 (K5) promoter (K5.Smad7) which expresses Smad7 transgene at a mild level (~2 fold of the endogenous Smad7 in the skin). These mice did not have overt skin defects as shown from our previous Smad7 transgenic mice expressing much higher levels of the Smad7 transgene (*EMBO J 2002, 21:2580–90*). K5.Smad7 mice from the above low expressor line and non-transgenic littermates were subject to 6-mm full-thickness excisional wounding. K5.Smad7 mice exhibited early scab rejection, reduced inflammation, and accelerated re-epithelialization as compared to non-transgenic mice. To further determine the stage-specific effects of Smad7 on wound healing, we generated a transgenic model in which Smad7 transgene expression can be induced in the epidermis and hair follicles (gene-switch-Smad7) by topically RU486 application. Smad7 induction from day 3 to day 7 after excisional wounding reduced inflammatory responses through suppressing expression of a variety of inflammatory cytokines/chemokines in gene-switch-Smad7 mice as compared to control mice. Meanwhile, overexpression of Smad7 exhibited accelerated re-epithelialization, which correlated with increased expression of metalloproteinases and elevated Erk (extracellular signal-regulated kinases) signaling in the leading epidermal edges in gene-switch-Smad7 wounds compared to control wounds. Smad7 induction from day 7 to day 20 after excisional wounding reduced dermal fibrotic response and angiogenesis in the dermis, resulting in a better tissue repair. We conclude that the effectsof Smad7 on wound healing are likely due to blocking the negative effects of TGFβ on cutaneous wound healing.

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FIBROBLAST GROWTH FACTOR – BINDING PROTEIN CDNA AND TRUNCATED VARIANTS ARE ACTIVE IN DIABETIC WOUND HEALING

Jayasri Dasgupta*, Michaela Bittner¶, Andreas Goppelt¶, Sabine Werner**, Hans-Dietmar Beer**, and Jeffrey M. Davidson*.
*Dept. of Pathology, Vanderbilt Univ. and VA Med Ctr., Nashville, TN; **ETH, Zurich; ¶SWITCH Biotech AG, D-82152 Neuried, Germany **(jayasri.dasgupta@vanderbilt.edu)**

Fibroblast growth factor – binding protein (FGF-BP) is a secreted protein that appears to function as a low affinity heparin –binding protein. FGF-BP binds to FGF-1 and -2 in a non-covalent, reversible manner to mobilize and solubilize these growth factors from their storage sites in the extracellular matrix. FGF-BP is involved in both developmental and adult tissue homeostasis as well as in angiogenesis and tumorogenesis involving FGF-1/2. FGF-BP is overexpressed in several tumor types: head and neck, skin, cervical, and lung cancer, squamous cell carcinoma, and colon and breast adenocarcinoma. To establish the effect of FGF-BP on wound healing, several forms of FGF-BP cDNA were administered by particle-mediated gene transfer into various animal wound models using the gene gun (Bio-Rad). In a rat incisional wound model, gene gun cDNA delivery of full length FGF-BP at the time of surgery produced a 117% increase of wound strength in diabetic rats at 10d, although the relative increase did not reach statistical significance ($P < 0.08$). Two truncated variants of FGF-BP (pFGFbp10 and 17) were also administered in the rat incisional wound model by gene gun technique. pFGFbp17 increased the wound strength in diabetic rat 129%($p < 0.03$), and the relative increase reach statistical significance ($P < 0.008$). In the rabbit ear ulcer model, particle-mediated transduction of full length FGF-BP increased collagen content by 195% and wound closure rate 38% at 10d post-surgery. These findings show that FGF-BP gene overexpression has a greater relative effect on wound healing in the diabetic rat model. The cDNA also had a significant effect in a rabbit excisional wound model that depended on granulation tissue formation. Truncated forms of the molecule may have higher therapeutic potency. FGF-BP has an important role in FGF-1/2 mobilization and macrophage functions, and FGF-BP gene therapy for wound healing can improve the process by stimulating angiogenesis, epithelization and collagen synthesis in target tissue.

Supported by SWITCH Biotech and Veterans Affairs.

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ADENOVIRAL OVEREXPRESSION OF MCP-2 INDUCES MACROPHAGES AND IMPROVES WOUND HEALING IN DIABETIC RATS

Y. Shi*, J. Liu*, M. Bittner#, A. Goppelt#, J.M. Davidson*,
*Vanderbilt Univ, Nashville TN; #Switch-Biotech, Neuried, Germany

The recruitment of leukocyte subsets is mainly driven by chemokines. Monocyte chemotactic protein-2 (MCP-2) belongs to the CC subfamily of chemokines, and it is especially a chemoattractant for monocytes. Of all CC subfamily members only MCP-2 interacts with multiple receptors, and its N-terminal truncated isoform is a natural chemokine inhibitor. Gene expression profiling in excisional wounds showed MCP-2 was down-regulated 40% in wild type mice and 69.2% in diabetic mice at 24 h after wounding. To determine the biological response to MCP-2 during wound healing, we constructed an adenoviral vector (Ad-MCP-2). Ad-MCP-2 infected cell conditioned medium contained 460 ng/ml secreted MCP-2 (ELISA) and had chemotactic activity similar to recombinant full-length MCP-2 but not to MCP-2 N-terminal 8–13aa peptides in a THP-1cell migration assay. In an incisional wound model in STZ induced diabetic rats, the breaking strength of Ad-MCP-2 infected skin wounds increased by 43% (10^8 PFU, $p < 0.05$) and 30% (10^7 PFU, $p < 0.05$) at 7d after Ad-MCP-2 injection compared with Ad-LacZ control. The wound closure strength of 10^7 PFU Ad-MCP-2 infected wounds still increased by 21% ($p < 0.05$) at 10d after injection. In normal rats, the breaking strength of Ad-MCP-2 and Ad-Lacz infected wounds was not significantly different. In rats, we injected either Ad-MCP-2 or Ad-LacZ (10^8 PFU) into each PVA sponge at 3d after implantation. Histological analyses revealed numerous ED-1 positive macrophages infiltrating in the experimental granulation tissue at 7d after Ad-MCP-2 injection. Our data provide evidence that MCP-2 improved wound healing in diabetic rats, and the recruitment of macrophages into the wound granulation tissues was the one of the mechanisms.

Supported by Switch Biotech and the Department of Veterans Affairs

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DETERMINATION OF BURN DEPTH USING NEAR INFRARED SPECTROSCOPY

K Cross[1], L Leonardi[2], J Fish[1], M Sowa[2] M Gomez[1], J Payette[2], M Hastings[2]
[1]University of Toronto, Toronto, ON; [2]NRC, Winnipeg, MB, Canada

Introduction: Burn depth, based on the hemodynamic alterations that occur following a thermal insult, can be assessed in a rapid, non-invasive, and nondestructive fashion using near infrared (NIR) spectroscopy. NIR has the capability to determine the difference between superficial and full thickness burn injuries.
Methods: Sixteen burn patients admitted to an adult regional burn center were studied and evaluated with the NIR point and imaging devices. Non-burned skin adjacent to the burn site was used as the control. NIR measurements were compared between superficial (8 wounds), full thickness (8 wounds) burn wounds and control sites.
Results: NIR was able to easily detect an increase in oxyhemoglobin (68.3%, $p < 0.05$), oxygen saturation (4.8%, $p < 0.05$%) and total hemoglobin (91.3%, $p < 0.05$) which typically occurs with superficial burn injuries. Full thickness injuries experienced a substantial drop in oxyhemoglobin (88.8%, $p < 0.05$), oxygen saturation (79.1%, $p < 0.05$) and total hemoglobin (77.5%, $p < 0.05$) in comparison to control sites.
Conclusions: These results confirm that NIR spectroscopy can successfully distinguish between superficial and full thickness burn injuries. The second phase of this study will involve determining the depth of indeterminant burn wounds and this preliminary data will also be presented.
Acknowledgement: National Research Council of Canada

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A CHRONIC PRESSURE SORE NUDE MOUSE MODEL

E. Wassermann[1], K. Gstaltner[2], H. Redl[1]
[1]LBI for Experimental and Clinical Traumatology, Vienna, Austria [2]Weisser Hof, Rehabilitation Center of AUVA, Klosterneuburg, Austria

The epidemiology of pressure sores is not clearly understood yet. Tissue layers involved in the pressure sores include skin, subcutaneous fat, deep fascia, muscle, and bone. Widely used experimental models lead to the pressure sores in the skin and the underlying fatty tissue only. The aim of the present study was to develop a clinically relevant deep pressure sore model in nude mice. METHODS: Six weeks old Balb/c nude mice weighing 25–30 g were used. After introduction of anesthesia a steel disk (Ø 5 mm, 0.5 mm thickness) was implanted under the musculus gluteus max.. The wound was closed with 4/0 sutures. The full recovery was allowed for 10 days. Animals with an insufficient healing were excluded. The pressure was induced using a Neodymium magnet (1.29 Tesla) in conjunction with the disk in un-anesthetized mouse periodically. A two hours compression was followed by one hour recovery for 4, 6 or 8 cycles, mimicking clinical setting. The controls underwent the same procedure, including the disk implantation but without placement of the magnet. RESULTS: The pressure sore depth was found to be cycle dependent – with the highest degree after 8 cycles. The histologic evaluations revealed signs of necrosis in the skin and subcutaneous fat after 4 and in the muscle only after 8 cycle of compression. PMN infiltration into the skin, subcutaneous fat, the deep fascia, and muscle was found in all groups. CONCLUSION: This model with clinically relevant magnitude and duration of pressure application leads to an injury in all tissue layers similar to the patient setting. This nude mouse model can be used to investigate the mechanism of pressure sores development and to study new therapeutic approaches (e.g. human cells) without the need of immunosuppression.
Acknowledgements: Lorenz Boehler Fond, 7/2002

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AGED RATS DISPLAY INCREASED ISCHEMIA-REPERFUSION SKIN INJURY IN THE *IN VIVO* MAGNET MODEL

David S. Rosenberg (d-rosenberg@md.northwestern.edu), Leonard Lu, Anandev Gurjala, Thomas A. Mustoe
Wound Healing Research Laboratory, Northwestern University, Chicago, IL USA

Introduction: Ischemia-reperfusion (IR) injury is commonly associated with numerous pathologies including pressure sores, venous stasis ulcers, and lower extremity diabetic ulcers. The purpose of this study is to further investigate the relationship between age and ischemia-reperfusion skin injury in a rat model utilizing magnets for the purpose of injury creation.
Methods: Magnets were designed for subcutaneous placement and calibrated such that a second magnet placed externally over them would cause compression that exceeds capillary perfusion pressure (ischemia). Removing the external magnet results in reperfusion of the skin. After placing subcutaneous magnets in aged and young Fisher 344 rats, repeated cycles of external magnet placement and removal were performed.
Results: Visual analysis of the skin revealed statistically significant greater areas of injury in the aged rats relative to their younger counterparts ($37.4 \pm 13.3\%$ vs. $24.1 \pm 14.8\%$, $P < .02$)
Conclusions: Aged rats demonstrate an increased degree of injury relative to their younger counterparts in response to ischemia-reperfusion injury. Future studies will attempt to delineate differences in the markers of IR injury (such as myeloperoxidase and vitamin E levels) in aged versus young rats, giving insight to the mechanisms responsible for the impaired wound healing seen in the elderly.

Funding source: NIH grant

GENE THERAPY

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FGF-BP IMPROVES WOUND HEALING THROUGH THE INDUCTION OF MORE COLLAGEN IN RATS

Y. Shi*, J. Liu*, M. Bittner#, A. Goppelt#, J.M. Davidson*,
*Vanderbilt Univ, Nashville TN; #Switch-Biotech, Neuried, Germany

The fibroblast growth factors are important regulators during wound healing. To quench their biological activities, secreted FGFs are tightly bound to heparan sulfate proteoglycans in the extracellular matrix (ECM). One of the approaches for releasing the active FGF from ECM involves the binding to an FGF binding protein (FGF-BP), which prevents FGF degradation and retains its activities. FGF-BP enhances the proliferation of fibroblasts through FGF-1 and -2 and of endothelial cells through FGF-2. To detect the biological function of FGF-BP in wound healing, we constructed an adenoviral vector containing murine FGF-BP cDNA (Ad-FGF-BP). Polyvinyl alcohol (PVA) sponges were implanted subcutaneously in rats. 10^7–10^8 PFU of either Ad-FGF-BP or Ad-LacZ (as local control) was injected into the sponges at d3 after implantation. At d4 after injection, Ad-FGF-BP infected sponges displayed much better organization of granulation tissue with the presence of more macroscopic hemorrhage than the local control. At d7 after injection, Ad-FGF-BP infected experimental granulation tissues in sponges showed more collagen deposition. The contents of collagen, protein and DNA in Ad-FGF-BP infected sponges increased 24.0% ($p < 0.05$), 11.3% ($p < 0.05$, d4) and 28.2% ($p < 0.001$, d7), respectively. Incisional wounding was performed in rats and 10^8 PFU Ad-FGF-BP or Ad-lacZ was intracutaneously injected along the wound margins. At 7d after injection, the tensile strength of Ad-FGF-BP infected wounds increased 41.1% ($p < 0.05$). Our data indicate that overexpression of FGF-BP can improve wound healing, and the inductionof more collagen in granulation tissue may be one of the mechanisms.

Supported by Switch Biotech and the Department of Veterans Affairs

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IK-B ADENOVIRAL GENE TRANSFER IMPROVES WOUND HEALING

Schreiber J, Efron P, Park J, Moldawer L, and Barbul A
Dep. Surgery, Sinai Hospital, Baltimore, and University of Florida College of Medicine, Gainsville, FL

Introduction: One of the earliest events in wound healing and scar formation is the inflammatory phase. Deficient or excessive inflammatory responses result in aberrant healing. NFK-b is a transcription factor that regulates key genes responsible for multiple intra-nuclear inflammatory cytokines. When bound to IK-B, an inhibitory protein, this factor is in an inactive state. We studied the wound healing effects of irreversibly inhibiting the activation of NFK-b by continuously infusing adenoviral activated IK-B.
Methods: Seventy-five male Sprague-Dawley rats underwent subcutaneous implantation of polyvinyl alcohol sponge-osmotic pump constructs. The pumps were filled with a solution containing a genetically altered adenovirus containing the DNA for continuously activated IK-B protein. 50 µl of viral transport media containing 5×10^9 PFU of virus were delivered to each individual sponges daily. Controls included pumps filled with normal saline in one group or filled with sham virus in another group. Sponges were harvested on days 1,3,5, and 7 post-implantation. The sponges were analyzed for TNF-α and nitric oxide (NO) levels, as index of inflammation, as well as for hydroxyproline (OHP) content at seven days, an index of collagen deposition.
Results: Treatment with the IK-B virus resulted in significantly higher levels of OHP after seven days when compared to normal saline and sham virus treated sponges (654 ± 81 vs 546 ± 109 vs 498 ± 123 µg OHP/100 mg sponge, $P<0.05$ by ANOVA). Wound fluid NO concentration was significantly lower in the IkB group after 5 days (38 ± 15 vs 55 ± 12 vs 71 ± 27 µM, $P<0.05$ by chi-square). There were no differences in sponge TNF-α concentrations on days 1,3, and 5.
Conclusions: Treatment of wound sponge granulomas with IK-B increases the amount of collagen deposition after seven days. Reduction of inflammation by inhibiting NF-KB may be a possible mechanism of action, as reflected in decreased NO wound content.

044

ADENOVIRAL GENE TRANSFER OF IL-10 DIFFERENTIALLY REGULATES HYALURONIC ACID SYNTHASE 1: IMPLICATIONS FOR SCARLESS WOUND HEALING

ED Kozin, SG Keswani, PW Zoltick, AD Gordon, AB Katz, M Herlyn and TM Crombleholme.
The Children's Hospital of Philadelphia, Philadelphia, PA, 19104

Introduction. We have demonstrated that wounds treated with adenoviral overexpression of Il-10 (AdIl-10) heal scarlessly, resembling fetal wound healing. Hyaluronic acid (HA) is elevated in fetal wounds. We hypothesized that IL-10 creates a permissive environment for scarless healing through the differential mediation of hyaluronic acid synthases (Has1–3).
Methods. Adult C57 mice ($n=24$) were pretreated with 5×10^8 PFU AdIL-10, AdLacz, or PBS. 48 hrs post-injection, 2-cm incisional wounds were created at the site of injection. Wounds harvested at 1, 3, and 5 days were analyzed for mRNA transcript levels of Has 1–3 and GAPDH via RT-PCR and band densitometry. Data expressed as signal intensity ratio relative to GAPDH ± SEM.
Results. The three Has transcripts were expressed after pre-treatment and wounding at day 1, 3, and 5. At day 1, there is no significant difference in Has mRNA levels. At Day 3, Has1 transcript levels are significantly decreased in AdIl-10 treated wounds vs. controls. (Il-10 0.28 ± 0.04; LacZ 0.53 ± 0.06; PBS 0.43 ± 0.43, $p<0.005$) At Day 5, there was a significant increase in Has1 transcript levels in AdIl-10 treated wounds compared to controls. (Il-10 0.82 ± 0.08; LacZ 0.41 ± 0.06; PBS 0.57 ± 0.03, $p<0.005$) At Day 3 and 5, there was no significant difference in Has2 and Has3 levels in AdIl-10 treated wounds vs. controls.
Conclusions. Adenoviral mediated overexpression of Il-10 results in the differential expression of Has1 at day 3 and 5. Has1 encodes for high molecular weight HA, which is associated with inhibition of angiogenesis and leukocyte infiltrates as observed in the HA-rich environment of fetal wounds. The effects of AdIl-10 may in part be due to the differential expression of Has1 creating a permissive environment for fetal-like scarless wound repair.

045

ADENOVIRAL hTERT TRANSFECTION RESULTS IN ALTERED PHOSPHO-ERK ACTIVITY IN HUMAN DERMAL FIBROBLASTS

Leonard Lu (leonardlu@aol.com)[1], David S. Rosenberg[1], Jon E. Mogford[2], Thomas A. Mustoe[1]
[1]Wound Healing Research Laboratory, Northwestern University, Chicago, IL 60611 USA
[2]Strategic Analysis, Arlington, VA 22201

Introduction: Telomeres are nucleoprotein structures at the ends of each chromosome. Due to the inability of DNA polymerase to replicate the full length of the chromosome, up to 50–200 base pairs of the telomere are lost during each successive round of cell division. In adult human somatic cells, telomerase is not active resulting in progressive loss of telomere length and entry into replicative senescence as observed in cell culture. hTERT is the catalytic subunit of telomerase, an enzyme which maintains telomere length. Transfection of human dermal fibroblasts (HDFs) by hTERT has been shown to reverse the senescent phenotype seen in aging HDFs *in vitro*. ERK (p44/42) is a MAP kinase which functions as a critical intermediary in the determination of cell growth and differentiation. Activation of ERK occurs through phosphorylation of threonine and tyrosine residues.
Methods: In order to delineate some of the cellular mechanisms by which hTERT functions, we treated adenoviral hTERT (Ad-hTERT) transfected HDFs with TGFB1, and assayed phosphorylated ERK activity by Western blotting.
Results: Ad-hTERT treated HDFs demonstrated a 2–3 fold increase in phospho-ERK activity. In addition, our preliminary findings show that Ad-hTERT transfected HDFs have increased TGFB1, TGFB1-Receptor I and II, and COL1A1 gene expression by real-time rtPCR.
Conclusions: Increased phosphor-ERK activity as well as increased TGFB1, TGFB1-Receptor I and II, and COL1A1 gene expression is seen in hTERT transfected HDF's. Further studies will focus on defining other intermediary changes resulting from Ad-hTERT tranfection.

Funding source: Geron Corporation

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CHARACTERIZATION OF WOUND REPAIR EFFECTS AFTER rAd-p21 TREATMENT

D. Looper, D.W. Kang, D-L. Gu, D. Maneval, M. Zepeda
Department of Pharmacology, Canji, Inc., San Diego, CA 92121

Excessive scarring is a significant clinical problem, resulting in both adverse tissue form and function and the goal of therapeutic interventions is to reduce and prevent excessive scarring. We have demonstrated that a recombinant adenovirus containing the cyclin-dependent kinase inhibitor p21 (rAd-p21), inhibited scar formation by blocking cell cycle progression and attenuated cell proliferation at the wound site (*Perkins et al 2002*). Recently, we have shown rAd-p21 specific antiproliferative effects on granulation tissue *in vivo* (*Gu, et al*, manuscript submitted). Safety parameters using rAd-p21 for anti-scarring may include effects on wound strength and dehiscence of the wound. We tested effects of rAd-p21 on wound strength *in vivo* using tensile strength as an endpoint and included comparisons with other clinically relevant antiproliferative agents. Specifically, rAd-p21 at doses from 1×10^7 to 3.8×10^{10} particle (PN) per incision was administered intradermally to linear rat incisions and assayed 14–28 days post treatment. rAd-p21 mildly reduced tensile strength at high doses ($\geq3\times10^{10}$PN), whereas low to moderate doses (1×10^7 to 1×10^{10}PN) had no effect. Interestingly, all rAd-p21 treated wounds regained tensile strength indistinguishable from vehicle control, 4 weeks after treatment, suggesting that rAd-p21 wounds recover with time. An adenovirus control vector, not containing a gene (rAd-Empty), showed subtle reduction of tensile strength that was only statistically significant at day 21. This suggests that delivery of rAd-Empty alone in the wound has little effect on wound strength. Triamcinolone at 5 mg/mL/wound, 5-FU at 10 mg/mL/wound, and low doses of MMC did not significantly reduce tensile strength, although high doses of MMC (0.2 mg/mL/wound) severely reduced tensile strength which failed to recover after 28 days. Morphological analysis of all groups revealed necrosis only in the MMC treatment. This data suggests that rAd-p21 may reduce the hyperproliferative status in excessive scar formation with minimal effects on wound strength.

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rAd-p21 SIGNIFICANTLY ATTENUATES GRANULATION TISSUE FORMATION AND SCAR THICKNESS *IN VIVO*

D-L. Gu, I. Atencio, D. Looper, D.W. Kang, D. Maneval, M. Zepeda
Department of Pharmacology, Canji, Inc., San Diego, CA 92121

Excessive scarring is a significant clinical problem. p21, a cyclin-dependent kinase inhibitor, blocks cell cycle progression and attenuates proliferation of various cell types. Our *in vitro* rAd-p21 dose response studies of primary human dermal fibroblasts showed a 3–80 fold reduction of cell proliferation as measured by BrdU incorporation at doses of 1×10^{8}-3×10^{9} PN/mL, respectively. Further, rAd-p21 at 3×10^{9} PN/mL showed a 2-fold less procollagen I (PIP) production when compared to control virus. These *in vitro* data demonstrate that rAd-p21 significantly attenuates fibroproliferation and procollagen production in the target cells. *In vivo*, a rat PVA sponge model was used. rAd-p21, at doses of 1×10^{9}, 1×10^{10} and 5×10^{10} PN was delivered into sponges that had previously been treated with a granulation tissue stimulator, rAd-PDGF-B. Results showed that sponges receiving rAd-PDGF-B alone had the highest % granulation fill (40–60% fill area). Sponges that were treated with rAd-PDGF-B for the first injection followed by rAd-p21 on a second injection showed a dose-dependent decrease in % granulation fill as rAd-p21 doses increased ($p<0.001$). On day 5 post-injection, IHC staining identified p21 protein expression only in sponges receiving rAd-p21. In addition, the quantitative measurement of cells labeled by BrdU or Ki67 demonstrated that rAd-p21 significantly attenuated cell proliferation when compared to rAd-PDGF alone in this model ($p<0.01$). In another *in vivo* experiment, a rabbit excessive scar model was used. rAd-p21, at a dose of 2×10^{9} PN/wound was intradermally injected into the rabbit ear wounds that had previously been treated with PDGF-BB protein. Preliminary data showed that scar thickness in rAd-p21 treated wounds was significantly decreased in comparison to wounds treated with the control virus ($p<0.05$). These data suggest that rAd-p21 is effective in attenuating scars in the rabbit ear excessive scar model. Our *in vitro* and *in vivo* data support the further exploration of rAd-p21 as a useful therapeutic candidate for hyperproliferative diseases in patients.

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RETROVIRAL DELIVERY OF A DOMINANT NEGATIVE TGF-BETA RECEPTOR II MITIGATES SCAR IN A RABBIT MODEL OF SCAR HYPERTROPHY

Russell R. Reid, MD PhD (rcube91298@yahoo.com), Nakshatra Roy PhD, Jon E. Mogford PhD, Hannah Zimmerman BS, Chung Lee PhD, and Thomas A. Mustoe, MD FACS

Wound Healing Research Laboratory, Division of Plastic Surgery, Feinberg School of Medicine, Northwestern University

Effective blockade of the pluripotent cytokine TGF-beta as a means of cutaneous scar reduction is a strategy with great potential. This desired effect may be achieved through the overexpression of mutant TGF beta receptors within the wound milieu. Our goal was to examine the effects of dominant negative mutant TGF-beta receptor II (dnTGFRII) protein expression in a well-established rabbit ear model of hypertrophic scarring. Serial injections of a retroviral construct encoding a truncated TGFβRII and the marker green fusion protein (pMSCV-rIIdn-GFP) were performed in 7mm punch wounds at day 10 and day 14 (two-day injection group) or day 8, 10, 12 (three-day injection group) post wounding. Delivery of a null vector (pMSCV-GFP) at the same time points served as a negative control. Histomorphometric analysis of wounds harvested at day 28 revealed a statistically significant reduction (33%) in the scar elevation index in 2-day treated and a more modest reduction in SEI (17.5%) in the 3-day treated arm compared to null-treated controls. Confocal microscopy confirmed stable transfection of the construct in both peri-wound tissue as well as rabbit dermal fibroblasts transfected in vitro. Optimization of this novel application in retroviral gene therapy could lead to effective anti-scarring strategies.

Supported by NIH Grant 5-ROI-GM63825–2

OXYGEN & OXIDATIVE STRESS

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LACTATE CONTROLS VASCULAR DEVELOPMENT IN WOUND HEALING

R. S. Aslam, H. Scheuenstuhl, S. Beckert, H. Hopf, Z. Hussain, T. K. Hunt
University of California San Francisco, USA
Phone: (415) 502-1746 Email: aslamn@surgery.ucsf.edu

INTRODUCTION: High concentrations of lactate approx. 10 mM are characteristic of wound healing. We have previously shown that lactate instigates release of VEGF from macrophages and endothelial cells. Our present study uses an in vivo murine Matrigel model to study how lactate enhances vascular development and repair.
METHODS: Matrigel is a reconstituted basement membrane complex, a liquid at 4 degree C. When injected subcutaneously, it reconstitutes as a gel. 30 mgs of finely divided polylactate polymer (DL-lactide-co-glycolide) was mixed with 1cc of Matrigel, and 2 injections were made into the dorsum of each of 60 (average 7/group), 6-month-old Swiss Webster mice. The gel implants were harvested at 3, 6, 9, 11 and 16 days post injection, and were fixed in buffered formalin or frozen. Cell migration and vascular development were assessed using H&E, anti-CD31 and anti-MAC3 antibody stainings for leukocytes, fibroblasts, endothelial cells, and macrophages. Cells and vessels were quantified microscopically.
RESULTS: Control implants (without polylactate) developed little or no inflammation or angiogenesis. Polylactated implants developed highly significant number of endothelial cells and recognizable vessels at 6 and 9 days. At 11 and 16 days, sizeable vessels with surrounding connective tissue were found. Though a moderate inflammation occurred in the lactate groups, no foreign body reaction was seen. Larger implants of high molecular weight instigated little inflammation and few vessels.
CONCLUSION: Slowly released lactate leads to the formation of new vessels. Our results provide support for further clinical applications of lactate polymer in wounds.

NIH/NIGMS Trauma Training Grant

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THE ANGIOGENESIS INHIBITOR ENDOSTATIN IMPAIRS WOUND HEALING AT TUMOR-INHIBITING DOSES

J Michaels, M Dobryansky, RD Galiano, KA Bhatt, R Ashinoff, DJ Ceradini, JP Levine, GC Gurnter
New York University Medical Center New York, NY, USA

Introduction: Angiogenesis is thought to be important for granulation tissue formation and for the delivery oxygen and other nutrients to the healing wound bed. We examined whether angiogenesis inhibitors (AI) such as endostatin decrease neovascularization in full-thickness wounds leading to impaired granulation tissue formation and delayed wound closure.
Methods: Endostatin at tumor-inhibiting doses (20 mg/kg/BID) was injected daily starting three days prior to surgery. Two full-thickness wounds were created on the mouse dorsum using a novel wound healing model developed in our lab. A second experimental group had topical VEGF (10 μg/QD) applied to these wounds. Both groups were compared to PBS-treated controls. Wounds were analyzed for closure time, granulation tissue formation, and wound vascularity using CD31.
Results: Endostatin-treatment delayed wound closure compared to control mice ($17.4d\pm1.51$ *vs.* 12.8 ± 0.89, $P<0.05$), resulted in decreased granulation tissue formation at all time points ($P<0.05$), and significantly reduced wound vascularity as measured by CD31+ vessel counts ($P<0.05$). VEGF application to the wound bed of endostatin-treated mice normalized wound closure despite endostatin treatment ($13.8d\pm1.1$ *vs.* $17.44d\pm1.51$, $P<0.05$).
Conclusion: Endostatin impairs wound angiogenesis, granulation tissue formation and delays full-thickness wound closure. Topical VEGF was able to reverse this effect and may represent a novel approach to improve wound healing in patients receiving AI. These findings may have serious implications for patients undergoing AI treatment that require surgery or who have wound healing complications.
Acknowledgements: This project received no outside funding.

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SUPEROXIDE REGULATES α-SMA VIA p-38 MAPK

J.H-C. Wang, G. Q. Wang

Mechanobiology Lab, MSRC, Department of Orthopaedic Surgery
University of Pittsburgh, PA, USA. *wanghc@pitt.edu*

This study was to investigate the effect of cyclic mechanical stretching on superoxide and nitric oxide production in human patellar tendon fibroblasts (HPTFs) and to establish the role of superoxide and p38 MAPK in stretching-induced α-smooth muscle actin (α-SMA) expression. When HPTFs were grown in deformable silicone dishes and subjected to 8% cyclic, uniaxial stretching, it was found that HPTFs markedly increased the production of superoxide and nitrite but not nitric oxide. And, cyclic stretching of HPTFs increased phosphorylation of p38 MAPK with increasing stretching magnitude. In addition, stretching duration also influenced the expression of phosphorylated p38 MAPK, where the dependence followed a pattern of cellular response in p38 MAPK activation consistent with previous studies using human mesangial cells and rat 3Y1 fibroblasts. For short stretching durations (~15 min), the expression of phosphorylated p38 MAPK increased rapidly but decreased for longer stretching durations. Furthermore, in HPTFs treated with superoxide dismutase (SOD), which converts superoxide anions to hydrogen peroxide and an oxygen molecule, levels of stretching-induced p38 MAPK phosphorylation and α-SMA expression decreased compared to stretched fibroblasts not treated with SOD. Similarly, cells treated with SB202190, which specifically inhibits p38 MAPK activation, also decreased α-SMA expression levels. Therefore, these results suggest that superoxide regulates stretching-induced α-SMA expression via p38 MAPK activation in HPTFs subjected to cyclic stretching conditions.

Supported by the Arthritis Foundation Award and NIH grant AR049921 (JHW)

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REDOX CONTROL OF DERMAL WOUND HEALING

Chandan K. Sen, Srikanth Pendyala, Savita Khanna, Thomas K. Hunt and Sashwati Roy.

Department of Surgery, Davis Heart & Lung Research Institute, The Ohio State University, Columbus, OH 43210

Reactive oxygen species (ROS) are generally thought to be deleterious to biologic systems. The phagocyte NADPH oxidase provides a classical example of deliberate ROS generation. High amounts of ROS are generated in transient bursts to kill pathogens. The recent discovery of another family of NADPH oxidases, the Nox/Duox family, provides additional examples of deliberate generation of ROS by non-phagocytic cells present at the wound site. Our working hypothesis is that ROS generated by wound-related cells (low-ROS by Nox; and residual ROS in the aftermath of phagocyte respiratory burst) support early-phase acute wound healing by inducing redox-sensitive signal transduction pathways especially those related to wound angiogenesis.
We have observed that ROS-sensitive redox signaling pathways drive wound angiogenesis. Genetic as well as pharmacological approaches to deliver low concentrations of ROS promote dermal wound angiogenesis, contraction and closure. While at such low levels ROS do not serve as a potent disinfectant, they may act as modulators of cellular response facilitating the healing process. Strategies to decompose ROS at the wound site impaired healing. Congenital defect in human NADPH oxidase results in impaired wound healing as well as increased susceptibility to infection. We have observed that NADPH oxidase deficient transgenic mice suffer from impaired healing even under super sterile conditions; the impairment is corrected by low-dose ROS delivery. Dermal wound healing is subject to tight redox control. The state of wound tissue oxygenation is a key factor that enables such control.

Supported by NIH-NIGMS 27345

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PSYCHOLOGICAL STRESS IMPAIRS HEALING AND OXYGENATION IN CUTANEOUS WOUNDS

Gajendrareddy PK[1, 2], Ilangovan G[3], Kuppusamy P[3], Horan MP[2], Eijkelkamp N[2] and Marucha PT[1, 2].
[1]Dept. of Periodontics, UIC; [2]Depts. of Oral Biology, and [3]Intern. Med., Center for Biomed. EPR Spectroscopy & Imaging, OSU.

Human and animal studies have shown that psychological stress impairs wound healing. Catecholamines released in response to stress could cause vascular changes that would restrict oxygen delivery to the healing wound. Hence, we hypothesized that stress impairs healing by disrupting oxygen balance in the wounds. 6–8 week, female SKH-1 mice were subjected to stress by confinement in well-ventilated 50-ml conical tubes. Stressing was done three days prior to wounding and five days post wounding, during the active cycle of the mice. Wounds were placed using a standard, 3.5 mm-diameter biopsy punch. Each mouse received two wounds on their dorsum, behind their shoulder blades. Wound oxygenation was measured by EPR oximetry. Gene expression was measured by real time PCR. Phentolamine, an alpha-adrenergic antagonist was used in conjunction with EPR oximetry to determine catecholamine-mediated modulation of wound oxygenation during healing. Our results on the effects of stress on wound healing were consistent with previous results in the model, and showed 19% – 30% larger wounds in the stressed animals when compared to controls. Oxygen levels in the wounds showed a 17.49 to 34.38% ($p < 0.001$) decrease in the stressed animals on days 1 through 5 post wounding. Furthermore, gene expression studies of a hypoxia driven gene, iNOS, showed an increase in expression on days 1 (205%; $p < 0.0003$), 3(96%; $p < 0.04$) and 5 (249%; $p < 0.0003$). eNOS expression increased by 66%($p < 0.02$) on day 1 post-wounding, with no significant difference in nNOS expression. Phentolamine ameliorated the effect of stress on wound oxygenation on day 1 post wounding. These findings show that stress-impaired healing is associated with reduced tissue oxygen and altered expression of a hypoxia-induced gene. Furthermoe, the data suggests a role for catecholamines in stress-induced impairment of wound oxygenation during early healing.

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HBO MEDIATES INCREASED NITRIC OXIDE PRODUCTION ASSOCIATED WITH WOUND HEALING

J.V. Boykin[1], C. Baylis[2], V. Sommer[1], L. Shawler[1], M. Watkins[1], J. Young[1], S. Louissaint[1], M. Crossland[1],
[1]Retreat Hospital Wound Healing Center, Richmond, VA [2]Dept. of Physiology, West Virginia Univ., Morgantown, WVA

Hyperbaric oxygen (HBO) is used adjunctively to treat chronic, non-healing wounds. Increased nitric oxide (NO) production during HBO is suggested as an important mechanism that promotes enhanced wound healing. The purpose of this study is the documentation of NO production during and following HBO treatments (txs). Diabetic and non-diabetic patients receiving HBO therapy (20 txs; 2.0ATA × 90 min) provided wound fluid and fasting plasma and urine specimens for nitrate (NOx) determinations. Nitrate measurements were obtained prior to HBO (baseline), after 10 and 20 txs, and at one and four weeks following HBO completion. In patients healing after HBO baseline plasma NOx ranged between 17.7 and 33.20 μM. At 10 txs plasma NOx increased >50% to between 35.8–43.5 μM. At 20 txs plasma NOx decreased to between 22.3–35.5 μM. Healing diabetic patient plasma NOx increased after HBO reaching 170% of baseline at one month (57.8 ± 0.15 μM). Plasma NOx of healing non-diabetic patients decreased to baseline levels at one month after HBO. Urine NOx values mirrored those of plasma NOx; wound fluid NOx elevations were delayed compared to plasma and urine. Conversely, non-healing HBO patients did not demonstrate the early, significant elevations of plasma NOx after 10 txs. This study documents, for the first time, an early (10 txs), significant elevation of plasma and urine NOx after HBO that is associated with successful healing in diabetic and non-diabetic patients. A similar early increase in plasma and urine NOx was not observed in patients not responding to HBO therapy. These findings suggest that HBO-mediated increased NO production plays a critical role in the correction of impaired wound healing in selected patients. Furthermore, early plasma and urine NOx determinations after HBO therapy may prove valuable in the prediction of the clinical outcome of treatment.

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THE EFFECT OF AN OXYGEN EMULSION ON COLLAGEN DEPOSITION IN SECOND-DEGREE BURN WOUND REPAIR

J. Li, Y.P. Zhang, L. Zhu, P.M. Mertz, A.L. Cazzaniga, C. Ricotti, P.J. Zalesky*, L.C. Hsu*, J. Creech*, W.H. Eaglstein, S.C. Davis.
University of Miami, Miami, FL, USA; * TherOx, Inc., Irvine, CA, USA* TherOx, Inc., Irvine, CA 92612, USA

A porcine model of second-degree burn wound was used to evaluate the effect of a newly developed topical oxygen emulsion (TOE) on collagen deposition during wound repair. A total of 6 pigs were used for the study. The burn wounds were treated with air exposure (no treatment), vehicle control or TOE, which contains super-saturated oxygen and releases oxygen in sustained high level when applied topically. Skin wound samples were collected at days 0, 1, 4, 7, 10, 14 and 21 after wounding. Semi-quantitative Reverse Transcription and Polymerase Chain Reactions (RT-PCR) were used to examine the mRNA expressions for type I and type III collagens and matrix metalloprotease-1 (MMP-1). RT-PCR products were run on ethidium bromide gel and analyzed under UV light with Bio-Rad Gel Document 2000 system. The expression intensity was recorded as the mean gray value. One-way analysis of variance was used for statistical analysis. The results showed: 1). Higher mRNA expressions of collagen III and MMP-1 were observed in TOE treatment group compared with air exposure and vehicle control groups. 2). MMP-1 expression increased shortly after wounding with peak at days 4 and 7. 3). Collagen III expression increased earlier with peak at day 10 and day 14. 4). Collagen I expression level increased later than that of collagen III. Significant increase was seen after day 10 with the highest at day 21. However, there was no significant difference between vehicle control and TOE treatment groups. The data suggests that sustained high level of oxygen release by TOE may promote wound repair through the mechanism of increased expressions of type I and type III collagens. The TOE also increased the expression of MMP-1, which might accelerate clearing damaged collagens in the earlier phase of wound healing and promote collagen remodeling in the later phase.

This study was supported by grants from DOD/DARPA and the Dermatology Foundation of South Florida.

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EPIFLUORESCENT AND LIGHT MICROSCOPIC VISUALIZATION OF BACTERIAL MICROCOLONIES IN ACUTE WOUND INFECTIONS – ARE THESE BIOFILMS?

Carlos Ricotti, Alex Cazzaniga, Halland Chen, Patricia M. Mertz, Stephen Davis

A biofilm is a formation of surface-associated microbial cells that are enclosed in a self produced extracellular polymeric substance matrix. This definition is acceptable for in-vitro research but a clear definition for biofilm-associated diseases has yet to be elucidated. A structural/morphological definition is currently used to define biofilms; additionally, physiological or molecular criteria will allow us to define biofilms in disease infection accurately. Our objective over the past several years has been to characterize and observe bacterial biofilms in wound infections using different wound models. To broaden our current understanding of biofilm morphology in wounds for this study we used epifluorescent and light microscopy to visualize wound pathogenic pseudomonas aeruginosa bacteria in a porcine partial thickness infection model.
Three experimental animals were used for this study. After animal preparation, partial thickness wounds were created on the backs of 3 animals. Wounds were then inoculated with 10^6 colony forming units/ml (CFU/ml) of *Pseudomonas aeruginosa* and covered for 48 hours to allow bacteria to colonize and infect the wound. Biopsies were obtained from normal skin, wounds before inoculation, wounds at 48 hours and wounds 48 hours after inoculation. Biopsies were processed and stained with Hematoxylin and Eosin for light microscopy and Calcofluor White and Ethidium Bromide for epifluorescence microscopy.
Images obtained using epifluorescent and light microscopy demonstrate that bacteria form aggregates of microcolonies. These structures are representative of bacterial biofilms and support the hypothesis that bacteria live as biofilms in wound infection. Although currently there is no established and accepted definition for biofilm associated diseases, we anticipate that more studies looking into physiological changes of these structures will clarify our current understanding of wound infection and treatment.

WOUND INFECTION

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IN VITRO EVALUATION OF BIOFILM DEVELOPMENT ON TISSUE ENGINEERED SKIN

CA. Charles[1], CA Ricotti Jr.[1], SC Davis[1], RS Kirsner, MD[1, 2, 3]
[1]University of Miami School of Medicine, Department of Dermatology and Cutaneous Surgery, [2]Department of Epidemiology and Public Health, Miami, Florida, USA, [3]Veterans Administration Medical Center, Miami Florida, USA

Bacteria reside in various forms, including planktonic, or free-floating, or as biofilms, which are a tightly adherent, potentially more treatment-resistant form. Biofilms, composed in part as polysaccharide matrices, can act as protection for bacteria by shielding them from antimicrobials and the innate immune system. It is estimated that bacterial biofilm are responsible for 65–80% of all chronic infections and may be responsible in part for non-healing of chronic wounds.
An advance in the treatment of non-healing wounds has lead to the development of human skin equivalents (HSE), which can successfully treat many non-healing wounds. Better understanding the interaction between bacteria and HSE might improve patient outcomes. Studies involving planktonic *E. coli* and HSE have demonstrated the growth of bacterial colonies only on the dermal surface. Growth was not seen on the epidermal layer of the HSE, possibly due in part to the expression of innate antimicrobial peptides called human β defensin-2 (hBD-2), which are expressed by HSE keratinocytes. The objective of this study was to determine if biofilm formation could occur on a bioengineered HSE.
We used a commercially available HSE. Three (3) mm full-thickness incisions were made in a triangular section of HSE. Each section was inoculated on the epidermal aspect with 1.0×10^5CFU per gram of Pseudomonas aeruginosa at 35 degrees Celsius for specified time points. Sections of HSE were sampled in areas of injury at various time points. Biopsy sections were processed for histologic analysis with H&E and epifluorescent microscopy to visualize *Pseudomonas aeruginosa*. We found that biofilm formation occurred at multiple time points on HSE. Colonies of adherent bacteria were visualized within the injured area of the epidermal layer of HSE by H & E staining. Epliflureosecnt microscopy using calcofluor white staining revealed the characteristic exopolysaccharide (EPS) matrix of biofilm.
Visualization of the expression of human β defensin-2 (hBD-2) with HSE after biofilm formation is underway. This knowledge will help to understand the role of bacterial biofilms within HSE and their effect on subsequent healing.

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SILVER DRESSINGS: EFFECTIVE ANTIMICROBIAL THERAPIES?

SC Davis, C Ricotti, AL Cazzaniga, PM Mertz.
University of Miami School of Medicine, Department of Dermatology & Cutaneous Surgery

Many silver-dressings have been recently developed to prevent infection of burn and chronic wounds. Antimicrobial therapies are usually studied in *in-vitro* assays, which evaluate the antimicrobial efficacy against free-floating bacteria. In a wound environment bacteria attach to tissues and establish bacterial biofilms. Biofilms are communities of colonies of bacteria and other microorganisms encased in a self produced exopolymeric substance. Biofilms help protect the bacteria from the environment and limit the effectiveness of antimicrobials. In this study we evaluated the efficacy of two silver dressings to eradicate biofilm-associated and planktonic *Pseudomonas aeruginosa* cells using our biofilm burn wound animal model.
Three pigs were used in this study. Second-degree burn wounds were made on one-half of the animal's back and inoculated with a burn wound isolate of P. aeruginosa. These wounds were then covered for 72 hours with a polyurethane dressing to allow biofilm formation. After 72 hours, additional burn wounds were made on the unwounded half of the animal. The new burns were then inoculated with the same *P. aeruginosa* strain. At this 72 hour point we had established two bacterial groups, one side of the animal had burns with planktonic bacteria and the other half with biofilm bacteria.
Both sides of the animal were treated 20 minutes after inoculation of the planktonic group with the following dressings: 1) Nanocrystalline Silver, 2) Hydrocolloid Silver, or 3) untreated. Wounds were cultured from all treatment groups at 24, 48 and 72 hours post treatment. Sites were cultured quantitatively using a novel flush-scrub technique to obtain both planktonic and biofilm bacterial counts. The baseline biofilm bacterial count was 7.56 LogCFU/ml (prior to treatment).
The hydrocolloid dressing significantly reduced planktonic bacteria counts as compared to untreated and nanocrystalline silver dressing at 24, 48 and 72 hours. However, biofilm bacteria counts for both dressings were similar to untreated control at all sample points. Our study demonstrates that both silver dressings showed limited effect against *P. aeruginosa* biofilm-associated cells when compared to untreated wounds.
Based on our results we question the effectiveness of silver dressings for infected wounds that are colonized with biofilm associated-cells. We conclude that anti-biofilm susceptibility models may improve on current antimicrobial sensitivity assays.

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EFFECTS OF TWO TOPICAL ANTIMICROBIAL AGENTS ON *STAPHYLOCOCCUS AUREUS* BIOFILMS IN A PORCINE MODEL

Davis SC, Cazzaniga AL, Ricotti C and Mertz PM.
Department of Dermatology and Cutaneous Surgery, University of Miami School of Medicine, Miami, Florida

Biofilms are a combination of microorganisms and extrapolysaccharide matrices. Our laboratory has previously demonstrated that biofilms are present in both acute and chronic wounds. Once biofilms are established, phagocytosis and diffusion of antibiotics are impaired thus contributing to antimicrobial resistance due to increased bacterial virulence. The purpose of this study was to determine the efficacy of two topical antimicrobial agents on partial thickness wounds containing *Staphylococcus aureus* biofilms. All wounds were inoculated with 10^6 CFU/ml and covered for 48 hrs under a polyurthene film to promote biofilm formation. Wounds were divided into three treatment groups; triple antibiotic ointment (Polymyxin B sulfate, bacitracin zinc, neomycin), mupirocin cream and untreated control. Wounds were treated twice daily. Wounds were cultured for bacterial quantitation at 24, 48, 72, 96, and 120 hrs. Significant reduction in CFU/ml was observed only after several days of treatments. This finding supports the antimicrobial resistance that occurs when bacteria live within biofilms. Our previous studies demonstrated that both of these agents were able to completely eliminate planktonic *S. aureus* (10^6) at the early time points. This study demonstrates that when bacterial biofilms are established in wounds there is a longer response time for topical antimicrobial activity suggesting bacterial resistance.

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THE EFFECT OF LOCALIZED INTRA-ABDOMINAL INFECTION ON COLON ANASTOMOTIC HEALING IN RATS

T. Kurt, A. Tatar, F. Vladimir, S. Cassaro, M. Cioroiu, R. Grossi
The Mount Sinai School of Medicine, Cabrini Medical Center, NY USA

Previous studies have demonstrated a decrease in intestinal healing in intra-abdominal infection. Currently, the effect of localized intra-abdominal infection on anastomotic healing and strength is not known. This study was designed to evaluate anastomotic wound healing in the presence of intra-abdominal abscess.
30 male Sprague Dawley rats weighing 270–300 grams were used for this experiment. 20 animals underwent cecal ligation and puncture (CLP) with 16 gauge needle. They were followed for 14 days. 9 of them died in the first week. The CLP animals had an initial 20% weight loss; however by day 10 they had a gradual return toward control weight. At the end of 14 days, rats from CLP group ($n = 11$) and control group ($n = 10$) underwent laparotomy and single-layer left colonic anastomosis. Blood cultures and abdominal swab cultures were sent from both groups. Post-operatively, animals were allowed to recover, food and water were offered *ad libitum*. At post-operative day five, animals were euthanized by thiopental overdose. Following anastomotic bursting pressure measurements, two samples were taken from the anastomotic line for subsequent determination of hydroxyproline content. Groups were compared with Students't test. Data were expressed as means $\pm$ SEM.
In the post-operative period, all rats lost 10% to 12% of their body weight. No bacterial growth was detected in cultures. Hydroxyproline content measurements were significantly higher in the CLP group (11.2 ± 0.3 vs. 9.8 ± 0.5 ug HPO/mg dry tissue weight. $P < 0.05$). On the other hand, no significant difference was observed in bursting pressure measurements between the groups (177 ± 6.3 vs. 164 ± 10.4 mmHg. $P > 0.05$).
These findings suggest that localized intra-abdominal infection without sepsis doesn't inhibit intestinal anastomotic healing.

Supported by a grant from Turkish Scientific and Technical Research Council.

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PREDICTORS OF POOR OUTCOME IN *S. AUREUS* WOUND INFECTION

L. Tibor, S.E. Cramton, H.W. Hopf, F. Götz, T.K. Hunt
University of Tübingen, Germany and University of California, San Francisco

Objectives: *Staphylococcus aureus* is the most common bacterium cultured from infected wounds. We hypothesized that diabetes mellitus (DM) and/or peripheral arterial disease would be patient predictors of severe outcomes, defined as amputation, sepsis, or death, and infections caused by resistant strains would be bacterial predictors of severe outcomes.
Methods: With IRB approval, 50 *S. aureus* strains (45 wound, 5 central line) were isolated during routine clinical care. Investigations included antibiotic resistance (>5 mcg/ml), and PCR for STAR elements. Chart review included wound type, location, relevant comorbidities, antibiotic resistance, and outcome.
Results:

	Outcome *n* (% *of outcome total*)		
	Severe	**All others**	**Total**
Diabetic	7 (70)	6 (17)	13 (29)
Non-diabetic	3 (30)	29 (83)	32 (71)
MRSA STAR groups	2 (20)	8 (23)	10 (22)
All other STAR	8 (80)	27 (77)	35 (78)
MRSA	4 (40)	8 (23)	12 (27)
MSSA	6 (60)	27 (77)	33 (73)
Total patients	10 (22)	35 (78)	45

No non-DM patient with arterial disease ($n = 6$) had a poor outcome.
Conclusions: The strongest predictor of outcome was the presence of DM. Patients with severe outcomes were slightly more likely to have MRSA infection, although only 33% of patients with MRSA had severe outcomes. Although STAR/*arsRBC* groups have been shown previously to predict antibiotic resistance, in this collection there was no correlation between these molecular markers and outcome. This study shows that the strongest predictor of clinical outcome in wound infection is patient-dependent, while bacterial factors appear to play a lesser role.

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THE IMPACT OF NOSOCOMIAL RESISTANT *PSEUDOMONAS* INFECTIONS IN A BURN UNIT

Edward E. Tredget, Heather A. Shankowsky, Jonathan P. Lee, Todd Swanson
University of Alberta Hospital, Edmonton, Alberta, Canada

Introduction: *Pseudomonas aeruginosa*, remains a serious cause of nosocomial infection and septic mortality in burn patients particularly when nosocomially acquired. Our purpose is to investigate the morbidity and mortality associated with nosocomial infection with an aminoglycoside resistant *Pseudomonas* and associated costs compared to a group of patients with similar severity of burn injury that did not acquire resistant *Pseudomonas* during hospitalization.
Methods: Using a TRACS burn database, patients treated at our institution with *Pseudomonas* resistant to gentamicin were identified and case-matched to controls for age (± 5 years), TBSA($\pm 5\%$), admission year (± 5 years) and presence of inhalation injury. Patients who died <48 hours after injury were excluded. Data examined included demographics, number of days to onset of positive *Pseudomonas* culture and gentamicin resistance, as well as antibiotic use and cost.
Results: 42 patients admitted to our unit between 1980 and 2001 were identified with gentamicin resistant *Pseudomonas*. Patients in the resistant *Pseudomonas* (*Ps*) group were similar in age (39.2 ± 3.2 vs 40.4 ± 3.3 years), TBSA (47.5 ± 3.7 vs $48.9 \pm 3.7\%$), extent of full thickness injury (37.0 ± 3.9 vs $30.3 \pm 3.2\%$) and presence of inhalation injury (62.8% vs 55.0%) compared to controls. There was a significant increase in the mortality rate in the *Ps* group (39.5 vs 5.0%, $p < 0.001$) (paired t test) compared to controls and the morbidity in terms of length of stay, increased in the *Ps* group (73.1 ± 13.2 vs 55.8 ± 8.1 days). Ventilatory days (22.6 ± 5.1 vs 8.2 ± 2.4, $p < 0.05$), number of surgical procedures (4.5 ± 0.6 vs 2.9 ± 2.5, $p < 0.05$), and amount of blood products used (packed cells 47.9 ± 7.8 vs 18.6 ± 3.3, $p < 0.01$) (platelets 10.5 ± 2.9 vs 0.5 ± 0.3, $p < 0.01$) were all significantly higher in the *Ps* group compared to control. Costs associated with antibiotic requirements were also significantly higher in the *Ps* group ($\$3{,}191.90 \pm 848.00$ vs $\$613.60 \pm 145.50$, $p < 0.01$).
Conclusions: Our data demonstrate that nosocomial infection in burn patients with aminoglycoside-resistant *Pseudomonas* is associated with significantly higher morbidity and mortality and cost of care. Increased resource consumption in terms of length of stay, number of surgical procedures, amount of blood products, and antibiotic costs did not prevent significantly higher mortality rates when compared to control patients who avoided infection with the resistant organism. Thus, prevention, identification and eradication of nosocomial *Pseudomonas* infection are critical for cost-effective, successful burn care.
Acknowledgements: Alberta Heritage Foundation for Medical Research, Firefighters' Burn Trust Fund of the University of Alberta Hospital

BIOMATERIALS/BIOENGINEERING

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THE HUMAN FOREARM BIOPSY MODEL FOR ACUTE WOUND HEALING

T. Serena, V. W. Li, W. W. Li, on behalf of the Wound Healing Cooperative Group

Wound Healing Cooperative Group, The Angiogenesis Foundation, Cambridge, MA USA

Background: The study of wound biology and effects of advanced modalities would benefit from a reliable human model for normal healing. Eaglstein et al. previously suggested that the forearm biopsy may be useful in acute wound healing studies (J Am Acad Dermatol 45:857, 2001). The Wound Healing Cooperative Group (WHCG) here validates this model in healthy individuals treated with topical growth factor therapy.
Methods: In our randomized, double-blinded study design, 20 normal healthy volunteers underwent four 6 mm biopsies of the flexor surface of both forearms. Biopsy sites were randomly assigned to a control arm (daily bacitracin) or to one of three treatment arms: i) rhPDGF-BB 0.01% gel (Q.D.), ii) rhPDGF-BB (Q.O.D.), iii) rhPDGF-BB (Q.D.×7 days followed by bacitracin alone daily). The wounds were examined, measured and photographed daily until complete healing was achieved. Adverse events were monitored. Rates of healing and time-to-complete closure were measured. Repeat biopsies of a subset of healing wounds were performed for gene microarray analysis.
Results: The forearm biopsy model allowed direct quantitative and qualitative comparisons of acute wound healing outcomes achieved by rhPDGF-BB regimens versus standard care alone. There were no infectious complications. Subject compliance was excellent with < 3% of visits missed.
Conclusion: The forearm biopsy model has several advantages in the study of acute wounds: it allows for comparison of topical agents in a controlled fashion; studies can be conducted easily with good patient compliance; and the forearm allows for repeat imaging and tissue harvesting for gene profiling during healing.
Acknowledgements: Funding from The Angiogenesis Foundation.

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PROLIFERATION AND GROWTH FACTOR EXRESSION OF HUMAN KERATINOCYTES ON MICROCARRIER BEADS

R. Williams[1], R.S. Rees[2], W.J. Lindblad[1]

[1]Dept. of Pharmaceutical Sciences, Wayne State University, Detroit, MI [2]Sec. Plastic Surgery, University of Michigan, Ann Arbor, MI

Human keratinocytes have been extensively used for the treatment of large body surface area burn injuries. We have studied the potential for treating non-thermal, chronic wounds with keratinocytes grown on microcarrier beads placed into porous retrievable bags. The current studies were undertaken to examine the growth characteristics of these cells on Cytoline-1™ microcarrier beads, and how this growth correlated with the expression of growth factors.
Human keratinocytes were expanded in Keratinocyte Basal Medium-2 with standard supplements (Clonetics, Inc.). The cells were trypsinized and added to Cytoline-1™ (Pharmacia Biotech) beads at 13.3×10^6 cells per mL pre-equilibrated beads. The beads were seeded into roller bottles and cultured in a humidified 37° C incubator for up to two weeks. Cell numbers were monitored by direct cell counting on the beads following hematoxylin staining; and by protein and DNA content. Aliquots of media were also assayed for levels of PDGF and VEGF by immunoassay (Quantikine®, R&D Systems).
Keratinocytes showed a lag in growth with a sustained decrement in cells per bead from 456 to 346 cells/bead by day 7. This was followed by a robust growth response to 551 cells/bead by day 11. These values correlated with total cellular protein (994, 738, and 1164 ug); and DNA content. The secretion of PDGF and VEGF by human keratinocyte under these conditions, did not directly correlate to the cell number but rather indirectly to the increase in cell number after the 7 days of stagnant growth. These results suggest that the vulnerary effect observed with human keratincytes on microcarrier beads is dependent on acculturation of the cells to the microbead environment, after which they begin to express elevated levels of growth factors important for healing.
Acknowledgments: Funding was provided by Keracure, Inc. through NIH grant R41 GM064064.

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IMPROVING INTEGRA™ "TAKE" WITH NEGATIVELY CHARGED METHACRLYLIC ACID BEADS

AA Eckhaus, MD, JS Fish, MD, MSc, FRCS(C), MV Sefton ScD

University of Toronto, Toronto, Ontario, Canada

Introduction: Integra™ has gained acceptance as a dermal replacement for large burns with limited donor sites. Integra™ vascularization takes between 14 and 21 days. Accelerating the vascularization of Integra™ could improve "take" rates and potentially decrease the obligatory waiting period between application and autografting.
Hypothesis: Application of negatively charged methacrylic acid (MAA) beads will enhance vascularization of Integra™
Methods Male Wistar rats (n = 11) had two 2×3 cm contra lateral dorsal full thickness wounds excised and grafted using Integra™. The MAA beads were applied under the Integra™ topically on the wound bed. Three experimental treatments were compared: Group 1- high-dose MAA beads, Group 2- low-dose MAA beads, Group 3- Integra only. Laser Doppler Imaging (LDI) to assess perfusion, as well as digital photography was done on days 7,10 and 14. Tissue samples for H + E and Factor VIII histological staining were collected on day 14.
Results: The average "take" was 99% ($p < 0.05$) for high-dose MAA beads, 98% ($p < 0.05$) for low-dose MAA beads and 82% in the Integra™ only group at day 7. At day 7 ($p < 0.01$) and day 10 ($p < 0.05$), the low-dose MAA group had significantly greater perfusion than the Integra™ only group. There were no statistically significant differences at day 14. Microvessel density (MVD) counts revealed a >40% increase in the number of vessels in both the low-dose MAA ($p < 0.05$) and high-dose MAA ($p < 0.05$) groups when compared to the Integra™ only group. There was no difference in LDI perfusion or MVD counts between low and high-dose MAA groups.
Conclusion: Factor VIII staining revealed enhanced angiogenesis in Integra™ treated with low and high-dose negatively charged MAA beads. Low-dose negatively charged MAA beads improved and accelerated the vascularization of Integra™ in this rodent model.
Acknowledgement: Materials and Manufacturing Ontario

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FIXATION OF AUTOLOGOUS SKIN GRAFTS WITHOUT STAPLES OR SUTURES – EXPERIMENTAL STUDY

R. Mittermayr[1], E. Wassermann[1], M. Thurnher[1], M. Simunek[2], H. Redl[1]

[1]LBI for Experimental and Clinical Traumatology, Vienna, Austria [2]Baxter AG, Vienna, Austria

The objective of the study was to evaluate the efficacy of skin graft fixation with fibrin sealant (FS) alone by means of spray application with a low setting time (thrombin concentration 4–5 IU/ml) in comparison to point suture fixation as a clinical reference method. Furthermore, the influence of two different quantities of FS were tested.
Material and Methods: On the dorsum of 6 male pigs four full-thickness wounds (8×4 cm) were excised in each animal. Randomly the four defects were divided into 3 groups (FS 0.05 ml/cm^2; FS 0.15 ml/cm^2 (Tisseel, Baxter AG, Vienna, Austria); Suture) and covered with autologous split thickness skin grafts. Outcome measurements included hematoma/seroma formation, graft dislocation, wound contraction, graft take rate and healed wound area. Observational time points were the 5th, 14th and 21st postoperative day.
Results: Hematoma formation on day of surgery was more pronounced in the suture group (FS 0.05 ml/cm^2 vs. suture, $p < 0.05$), similar the situation on the 5th postoperative day but without statistical significance. Graft dislocation revealed marked but not significant extended area in the suture group vs. the FS 0.05 ml/cm^2 group. The FS 0.05 ml/cm^2 graft take on day 5 was found to be enhanced in comparison to the suture group. Excellent wound healing was notable on final observation day in the FS 0.05 ml/cm^2 group with a healed wound area of 99.7 (3.6/0.3). Corresponding values in the FS 0.15 ml/cm^2 was 96.9 (4.7/2.1) and 95.9 (2.7/2.1) for the suture group. Values represents median (Q1/Q3).
Conclusion: The application of a thin layer of FS (0.05 ml/cm^2) in a low setting rate (4–5 IU thrombin) in split thickness skin transplantation via a spray device shows comparable results to those yielded with suture point fixation (surrogate for staples). Advantages are e.g. better cosmetic results and initial hemostasis with minimizing the incidence of early hematoma formation. Equal properties of manufactured low thrombin (4 IU; Canada) preparation vs. 500 IU (US) diluted to a 5 IU thrombin solution was also demonstrated.

067

REVERSAL OF ϵ (γ-GLUTAMYL) LYSINE CROSS-LINKING AND DOWN-REGULATION OF FIBRONECTIN AND TISSUE TRANSGLUTAMINASE (tTGase) ACTIVITY IN HYPERTROPHIC SCARS FOLLOWING TREATMENT WITH 0.8% 1,4 DAB 2HCL

B. Hardas[1], K. Dolynchuk[2], C. Jacob[1], M. Griffin[3], C. Panchal[1].
[1]Procyon Biopharma Inc., Montreal, QC, CAN [2]Dept. of Plastic Surgery, University of Manitoba, Winnipeg, MB, CAN [3]Nottingham Trent University, Nottingham, UK

Hypertrophic scar formation is an unfavorable condition which is difficult to predict, prevent or treat. Although much research has been done on understanding hypertrophic scar formation, the exact underlying molecular mechanism has not been fully elucidated.
Hypertrophic scars younger than 6 months are known to over-express tTGase. It has been shown that treatment of hypertrophic scars with topical 1, 4 DAB 2HCl inhibited ϵ (γ-glutamyl) lysine cross-linking[14].
In the current study, 12 paired scar biopsies, either treated or untreated with 1, 4 DAB 2HCl were examined for the presence of ϵ (γ-glutamyl) lysine cross-linking by fluorescence immunohistochemistry. *In situ* tTGase enzyme activity, expression of latent tissue TGF-β binding protein-1 (LTBP-1) and fibronectin were also examined. Scars showed a marked reduction of ϵ (γ-glutamyl) lysine cross-linking following treatment with 1, 4 DAB 2HCl. Although the treated samples did not show any change in expression of tTGase, its *in situ* activity was noticeably reduced. Treated samples also demonstrated down-regulation of fibronectin and LTBP-1. Results suggest that topical treatment of hypertrophic scars with 1,4 DAB 2HCl not only reduced ϵ (γ-glutamyl) lysine cross-linking but also reduced tTGase activity, expression of fibronectin and LTBP-1[25] which are known to play a role in extracellular matrix storage of transforming grown factor-β (TGF-β), responsible for wound healing and scar formation.
[4]Dolynchuk KN. Wound Rep Reg 1996; 4(1): 16-20.
[5]Verderio E, Gaudry C et al. J. Histochem 1999; 47(11): 1417-1432.

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PHOTORESPONSIVE HYDROGEL SCAFFOLDS IN CUTANEOUS REPAIR

F.M. Andreopoulos[1, 2], I. Persaud[1], K.M. Gattas-Asfura[3], R.M. Leblanc[3]
Departments of [1]Biomedical Engineering, [2]Surgery and [3]Chemistry, University of Miami, Miami, FL

Bioactive scaffolds impregnated with multiple growth factors that are released at controlled rates, offer the possibility of enhancing the healing process by mimicking the work of the natural tissue. We have developed photoresponsive and biodegradable hydrogel matrices based on natural and synthetic polymers that can undergo phase transformations upon exposure to alternating wavelengths of irradiation. Photo-polymerization provides an effective and benign method for *in situ* hydrogelation with spatial and temporal control of the polymerization reaction. Polyethylene glycol, heparin and gelatin molecules have been modified at high yields with photosensitive groups that can be rapidly and reversibly photocrosslinked to hydrogel scaffolds in the absence of initiators. We are currently exploring the efficacy of these scaffolds as depots for the controlled delivery of basic fibroblast growth factor in cutaneous regeneration. We have demonstrated that the stability of b-FGF is enhanced by its incorporation within the gel matrix and its complexation with components of the scaffold (i.e. heparin) while the releasing peptide maintains its activity. Cell proliferation and partial thickness wound studies demonstrated that b-FGF could be affectively released from the scaffolds at controlled rates and its wound healing properties were a function of the mode of delivery. Rate changes up to 60% on the kinetics of peptide release could be easily achieved by altering the exposure times (seconds to minutes range). Similarly, we were able to alter the release profile of b-FGF by adjusting the ratio of the photosensitive polymeric macromers prior to crosslinking. These results suggest that the proposed photoresponsive gels scaffolds could serve as bioactive peptide delivery vehicles in a number of tissue engineering applications.
Acknowledgements: Stanley Glaser Foundation and the Departments of Biomedical Engineering and Surgery, University of Miami.

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SCAR REDUCTION BY ADHESIVE PATCHES: EFFICACY AND POTENTIAL MODE OF ACTION

Meyer-Ingold, W., and Achterberg, V.
Beiersdorf AG, 20245 Hamburg, Germany

There is a wide spectrum of treatment modalities to manage hypertrophic scars, techniques like surgical excision, intralesional application of corticosteroids, pressure therapy or laser therapy are extensively used.
From a cosmetic and physiological point of view scars are predominantly a concern of the patients, the cosmetic relevance for doctors is often of less importance. Therefore a treatment that can be performed by the patient her-/himself might be advantageous if it is efficacious and safe. Since the early 1980s so-called "silicone gel sheeting" has been a widely used safe clinical management option for hypertrophic scars and keloids.
The principle of the latter treatment has now been adapted and optimised towards a specific easily applicable adhesive polyurethane patch. This breathable, hypoallergenic patch is very well tolerated and avoids – in contrast to silicone sheets – moisture accumulation on the skin. Several clinical studies on both mature scars and on scar formation after surgical incisions show a reduction in visibility, redness, and roughness of the scars. The recommended eight weeks treatment might even be interrupted daily for twelve hours; the scar reducing effect is achieved as well.
Extensive investigations on the polyurethane patch in vivo and in vitro propose an improved remodelling process of the scar tissue due to thermal and pressure effects of the patch but not by stratum corneum hydration. In vivo a better microcirculation can be observed in the scar tissue after treatment and in vitro an activation of enzymes involved in the remodelling process can be measured.
In summary, physical effects are likely to be responsible for the successful results in clinical studies and individual applications of the new polyurethane patch for the reduction of hypertrophic scars.

EPITHELIZATION

070

EPIDERMAL WOUND HEALING IS DELAYED IN STREPTOZOTOCIN INDUCED DIABETIC PIGS

P. Velander, D. Hoeller, C. Theopold, R. Gheerardyn, O. Bleiziffer, F. Yao, E. Eriksson
Laboratory of Tissue Repair and Gene Transfer, Harvard Medical School, Boston, MA, U.S.A

Streptozotocin-induced diabetes is a well established model of diabetes in rodents. However, these small mammals differ from humans in a number of anatomical and physiological ways, in contrast to pigs, who are more similar to humans. No diabetic pig model has been used for the study of wound healing. Our hypothesis is that re-epithelialization would be delayed in the streptozotocin induced diabetic pig.
Method: Diabetes was induced by injecting Streptozotocin into two three month old female Yorkshire pigs. 52 full thickness wounds were created on the dorsum and dressed with polyvinyl chambers to keep the wounds wet and to allow for wound fluid monitoring. Serum glucose and wound fluid glucose concentrations were monitored daily. Wound contraction was monitored and biopsies taken on multiple days for re-epithelialization measurements.
Results: The serum glucose was significantly increased for the duration of the experiment (>350 mg/dl). Wound fluid glucose closely followed serum glucose concentration. There was no statistical difference between the contraction rates of wounds in diabetic pigs and healthy pigs. Re-epithelialization was significantly delayed in diabetic wounds.

Reepithelialization	Day 12	Day 14	Day 16
Diabetic pig	38%	59%	82%
Healthy pig	96%	100%	100%

Conclusion: Epidermal regeneration of full thickness wounds is significantly delayed in the Streptozotocin induced diabetic pig. This is a very useful model of impaired wound healing because of the close similarity to human skin. The wound chambers add the advantage of monitoring and manipulating the wound environment.

NIH Grant #2ROIGM5 144905AI

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TGF-ß-MEDIATED PARACRINE REGULATION OF KERATINOCYTE WOUND CLOSURE

A. Sharif-Afshar, A. E. Gorska, E. G. Neilson, J. M. Davidson, H. L. Moses, N. A. Bhowmick
Vanderbilt University Medical Center, Nashville, TN, USA

The importance of stromal-epithelial interactions in wound healing is well established. These interactions likely involve autocrine and paracrine action of multiple growth factors, including members of the TGF-ß family. TGF-ß1, ß2 and ß3 isoforms signal by sequentially binding to the TGF-ß type II and type I receptors, respectively. We address the role of TGF-ß signaling in dermal fibroblasts using a conditional fibroblastic TGF-ß type II receptor knockout mouse model (termed FßKO). We found that the loss of TGF-ß signaling in the dermal fibroblasts results in accelerated excision-wound closure compared with similar wounds in wild type mice. The mechanism of the altered rate of re-epitheliaization in the FßKO mice was examined with regard to keratiocyte motility and proliferation. The migration of keratinocytes through collagen I coated 8 μm pore filters in the presence or absence of fibroblast-conditioned media was tested. These experiments showed increased keratinocyte migration when incubated with FßKO dermal fibroblast conditioned media compared to media conditioned in wild type fibroblasts. Immuno-histochemical staining of paraffin embedded intact skin indicated both wild type and FßKO mice had similar low levels of keratinocyte proliferation, based on Ki67 staining. In healing wounds, only the distal wound edges of wild type mice were proliferative. In contrast, the FßKO mice exhibited elevated proliferation across the length of the wound, including the leading edge of epithelial closure. Together our results suggest TGF-ß signaling by the dermal fibroblasts suppresses re-epithelialization of excision wounds by regulating keratinocyte motility and proliferation through paracrine mechanisms.

Funding: DOD BC99184 and NIH CA85492.

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FULL-THICKNESS WOUNDING OF THE MOUSE TAIL AS A MODEL FOR DELAYED WOUND HEALING

V. Falanga,[a,b,c] D. Schrayer,[a] J. Cha,[a] Janet Butmarc[a], P. Carson[a], A.B. Roberts,[d] S-J S. Kim,[d];

[a]Roger Williams Medical Center; [b]Department of Dermatology and [c]Biochemistry; Boston University; [d]National Institutes of Health, Bethesda, MD

Experimentally induced wounds in animal models are useful in gaining a better understanding of the cellular and molecular processes of wound healing and in the initial evaluation of the safety and effectiveness of potential therapeutic agents. However, studying delayed healing has proven difficult in animals, whose wounds heal within a few days. In this report, we describe a novel method for establishing mouse wounds that require up to more than three weeks for complete closure, and we show the validity of this model in Smad3 null mice, which are known to display accelerated healing. Full-thickness wounds, measuring 0.3 by 1.0 cm, were made down to fascia on the dorsal aspect of the mouse tail in Smad3 KO mice and control littermates, approximately 1 cm distal to the body of the animal. The wounds were left to heal by secondary intention and were assessed histologically by computerized planimetry for wound closure at various times after wounding. These wounds in wild-type mice displayed delayed healing, with full closure occurring between 14 and 25 days after wounding. Complete closure of similar wounds in Smad3 null mice healed 30% faster ($p < 0.01$). By immunostaining with ki67, a marker for proliferation, Smad3 null animals also showed increased proliferation of dermal wound cells. Cultured dermal fibroblasts from Smad3 null mice showed increased baseline DNA synthesis and, interestingly, enhanced response to TGF-β1. By Western blot analysis, Smad3 null mice fibroblasts showed a compensatory increase in MAPK phosphorylation in response to TGF-β1, suggesting that MAPK overcompensation together with loss of Smad3 may be involved in the modulation of faster healing. We conclude that this novel tail wounding model can be useful for studying delayed or prolonged wound closure.
Experimentally induced wounds in animal models can be useful in gaining a better understanding of the cellular and molecular processes of wound healing. Such models have also proven themselves valuable in the initial evaluation of the safety and effectiveness of potential therapeutic agents targeted for chronic non-healing wounds. (Gottrup, Agren et al. 2000). However, no ideal animal model exists which reliably reproduces delayed healing. In the mouse, a mammal whose genome has been completely cloned and which is easily manipulated genetically, wounds normally heal within a few days, and with a great deal of contraction. (Morris, Wu et al. 1997; Gottrup, Agren et al. 2000) There are models utilizing either genetically altered and inbred mice with certain characteristics that cause delayed healing. (Carmeliet 1995) However, it would be useful to have wound healing models that are applicable to most wild type mice used as controls and which would have a large enough window of observation before healing occurs. In this report, we describe a novel method for studying delayed healing in mice. This method utilizes full-thickness wounds made down to fascia on the dorsal and mostly hairless aspect of the mouse tail. The wounds are left to heal by secondary intention and assessed histologically by computerized planimetry for wound closure at various times after wounding. In this first report, the validity of the model was determined by studying control littermates and Smad3 null mice, which have been shown to display accelerated healing. The results shown here suggest that this is a useful model for studying delayed healing in mice.
Methods
Animal protocol
Smad3 null mice were generated as previously described by targeted disruption of the Smad3 gene by homologus recombination
Work supported by NIH grants AR42936 and AR46557

073

EXPRESSION OF CASPASES 3, 9, AND 14 IN THE EPIDERMIS OF EARLY WOUNDS

M.K. Kuechle, M.L. Usui, R.A. Underwood.
University of Washington, Seattle, WA USA

Caspases are a family of at least 14 highly conserved aspartate proteases that are the major effector arm of apoptosis or programmed cell death. Previous immunohistochemical and biochemical studies have implicated two of the family members, caspase 3 and caspase 14, as having a role in the process of epidermal terminal differentiation. Reepithelialization of epidermal defects after wounding requires coordination between signals that initiate growth and migration as well as signals that initiate differentiation. We therefore examined the expression of caspase 3, caspase 9, and caspase 14 in the epidermis of early (up to 48 hour) human wounds. Two excisional wounds were created on the inner arm using a 3 mm biopsy punch and allowed to heal via secondary intention. Wounds were excised at 24 and 48 hours using a 6 mm biopsy punch, placed in OCT, frozen, cryosectioned and immunohistochemically labeled using affinity purified antisera for caspases 3, 9 and 14. At 24 hours, the distribution of caspase 3 and caspase 9 expression was unchanged from non-wounded skin. Caspase 3 was found in the upper differentiating cells of the granular layer, while caspase 9 was expressed in scattered dendritic cells in the epidermis. At 48 hours, caspase 3 expression was lost in the epithelial tongue, while caspase 9 expression was found in keratinocytes at the tip of the migrating tongue. Caspase 14 expression at 24 hours was noted at the tip of the migrating epithelial tongue, while at 48 hours, the normal expression of caspase 14 in the granular layer was lost. These results suggest that in 48 hour wounds, loss of caspase 3 and 14 expression in the migrating epithelium reflects the activated state of this tissue. The onset of caspase 9 expression at 48 hours in the leading edge of the wound suggests a role for caspase 9 in wound remodeling, as caspase 9 is known to participate in tissue remodeling during development. Characterization of the caspases involved during wound healing may lead to future therapies utilizng caspases or caspase inhibitors in situations of poor or aberrant wound healing.

074

WOUND HEALING ENHANCEMENT BY PULSED ELECTROMAGNETIC FIELDS

M.J. Callaghan, E.R. Kinnuncan, J. Michaels, B.J. Simon, G.C. Gurtner

The effects of pulsed electromagnetic fields (PEMF) on soft tissue are not well characterized. This study utilized a diabetic wound model to examine the effects of PEMF effects on soft tissue healing.
Methods: Wounds were created on the dorsum of db/db and wild type C57BL6 mice. Mice were exposed to PEMF (4.5 ms pulse/15 hz) for 8 hrs/day for 14 days. Gross closure was assessed with digital analysis of area changes over time. Histological examination assessed granulation and epithelial gap, cell proliferation (BrdU), and endothelial cell density (CD31). Human umbilical vein endothelial cells (HUVECs) were incubated in the presence or absence of PEMF for 8 hrs and VEGF/FGF2 was measured in culture supernatants by ELISA.
Results: Mice exposed to PEMF had accelerated wound closure at day 7 (wound area as % of original, db/db: 60% (PEMF) vs. 78% (control), C57BL6: 15% vs. 42%, $p < 0.05$) and day 14 (db/db: 21% vs. 55%, C57BL6: 8% vs. 28%, $p < 0.05$), with increased granulation and cell proliferation (db/db day 7: 52 ± 8 vs. 31 ± 5 cells/HPF (200x)). Immunohistochemical analysis revealed significantly higher CD31 density in wounds exposed to PEMF at day 7 (vessels/HPF, db/db: 28 ± 4 vs. 17 ± 4, C57BL6: 41 ± 7 vs. 28 ± 6) and day 14 (db/db: 32 ± 6 vs. 21 ± 5, C57BL6: 48 ± 5 vs. 40 ± 5). HUVECs in PEMF exhibited 5-fold higher levels of FGF2 compared to controls after 30 min ($20.50 \text{ pg/ml} \pm 6.75$ vs. $4.25 \text{ pg/ml} \pm 0.75$), with no change in VEGF through 8 hrs.
Conclusions: PEMF accelerates closure time and endothelial cell proliferation in wound healing. Upregulation of FGF2 in HUVECs exposed to PEMF suggests that release of angiogenic growth factors may explain increased vascular density and accelerated wound closure. Clinical uses include treatment for diabetic ulcers and other non-healing wounds.

This work is supported in part by the Alumni Fund, Alumni Association of SUNY Downstate College of Medicine (MJC).

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WOUND HEALING IS DELAYED IN WOMEN AND IN THE AGED: A POTENTIAL ROLE FOR THE HPA AXIS

Marucha PT[1], Engeland CG[1], and Cacioppo JT[2].
[1]Dept Periodontics, University of Illinois at Chicago; [2]Dept Psychology, University of Chicago

Both senescence and psychological stress have been shown to alter numerous immune components including inflammatory processes. However, whether such effects can be dissociated by gender is largely unknown. To determine the effects of gender and age on wound healing, a 3.5 mm round wound was placed under local anesthesia on the hard palates of 88 younger (18–35 years) and 56 older (50+ years) male and female volunteers. Immediately prior to wounding and 15, 30 and 60 min afterwards, blood was drawn from which cortisol and ACTH levels were determined, as both are immunosuppressive. Wounds were photographed and measured daily until healed. Preliminary analyses revealed that older individuals healed 25.6% slower than younger individuals ($P < .001$), and women healed 14.0% slower than men ($P < .05$). These results may have involved alterations in HPA activity, as blood work revealed significant differences in baseline hormone levels: cortisol levels were lower in the older group ($P < .05$), and ACTH levels were lower in females ($P < .01$). Furthermore, in women only, faster healing rates were related to both increased anxiety at the time of wounding ($P < .05$), and higher cortisol levels at all time points ($P < .01$). Moreover, preliminary results indicated that, in women, higher anxiety was also related to lowered release of IL-1β in the first 24 h. In men these relationships were not evident, although higher ACTH levels were related to faster healing times ($P < .01$). Taken together, these findings indicate that aging delays oral wound healing and provide evidence for a previously unreported gender difference in wound healing rates in favor of males. Furthermore, the effects of anxiety on wound healing seem to be sexually differentiated and transient activation of the HPA axis, especially in women, appears to be associated with healing rates.

(Supported by NIH P01AG16321)

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MECHANICAL STRAIN INDUCES HYPERTROPHIC SCARS IN MICE BY REDUCING STROMAL CELL APOPTOSIS

KA Bhatt, MR Greives, RD Galiano, RL Ashinoff, RG Bonillas, DJ Ceradini, DM Jones, JP Levine, GC Gurtner.
NYU Plastic Surgery.

Intro: Pathophysiology of hypertrophic scar formation remains unclear, potentially involving mechanical strain, burns, or infection. We sought to specifically examine the role of mechanical strain in hypertrophic scars. To do so, we developed and characterized a novel murine model which uses mechanical strain to produce hypertrophic scars in mice.
Methods: Paired incisions were created on C57BL6 mice (n = 20). After closure, mechanical strain was applied across one wound using a novel device; the other wound was a control. Starting at day 3, mechanical strain was increased every other day over 4 wks. Wounds were harvested each week, and examined histologically using Sirius red, DAPI, BrdU for proliferation, and caspase 3 for apoptosis.
Results: Strained wounds showed features of hypertrophic scars: raised borders, loss of rete pegs and adnexal structures in the epidermis overlying scars, blood vessels that were perpendicular and fibrillary collagen that was parallel to skin surface. Features persisted beyond 6 months. Wound collagen deposition in strained wounds increased 6-fold at 1 wk and over 12-fold at 2–4 wks, in parallel to an increase in number of stromal cells within scar (28-fold). This resulted in an increase in number of cells per unit area of collagen (over 42%). In all wounds, there was no significant difference in proliferation. There was a significant reduction in blood vessel (4-fold) and fibroblast (3-fold) apoptosis at 1–4 wks in strained wounds.
Conc: Mechanical strain alone is sufficient to produce hypertrophic scars in this model that are indistinguishable from human scars. Notably, hypertrophic scars appear to result from a marked reduction in apoptosis, rather than from a significant proliferation and upregulation of cellular collagen deposition, validating previous in vitro studies.

STEM CELLS

077

HUMAN MESENCHYMAL STEM CELLS INTERACT WITH KERATINOCYTES AND SUCCESFULLY ACCELERATE WOUND HEALING

Sadanori Akita[1], Hiroshi Nakagawa[1], Masashi Fukui[1], Kozo Akino[2],
1: Division of Plastic and Reconstructive Surgery
2: Division of Anatomy and Neurobiology Nagasaki University, Graduate School of Biomedical and Sciences, Department of Developmental and Reconstructive Medicine, Nagasaki, Japan

Human mesenchymal stem cells (hMSCs) obtained from a single donor from an iliac crest, were investigated with cutaneous wound healing models using nude rat, eliminating T cell-mediated immune reaction to the grafted stem cell of human origin. Co-culture of the human keratinocytes and the hMSCs revealed tight basal membranous interaction by electron microscopy.
$1.5 \times 1.5\ cm^2$ dorsal full-thickness defects including panniculus carnosus of F344/NJCl-rnu nude rats were covered by bi-layered porcine-derived collagen sponge artificial skin substitutes, Pelnac®, Johnson & Johnson, Tokyo, Japan, impregnated with 5×10^6 hMSCs grated to along with 0, 1, 10, or 100 μg of recombinant human basic fibroblast growth factor (bFGF). The tissues were harvested at 3, 7 42 days after grafting. The defects were remarkably epithelialized by 7 days after coverage with artificial skin substitutes and 10 μg of bFGF, while artificial substitutes with no hMSCs or artificial skin substitutes with hMSCs alone did not demonstrate such healing. The architectures of the artificial skin substitutes were integrated by day 42 in hMSCs-treated groups. The artificial skin substitute at least plays a role in as a template or scaffold of the grafted hMSCs. Up to day 3, the mesenchymal stem cell surface markers such as CD 29 and CD 44 were remained immunopositive in the groups with hMSCs-treated groups over the reconstructed dermis-like tissue. By 42 days after grafting, artificial skin substitutes with hMSCs and 10 μg of bFGF demonstrated the total epithelization and the keratinocytes by this treatment exhibited the pan-cytokeratin of human origin by immunohistochemical expressions. Thus, the hMSCs with bFGF treatment using an artificial skin substitute as a successfully heal. These results suggest that the human mesenchymal stem cells may be utilized for wound coverage together with bFGF and artificial skin substitutes.

Type acknowledgements here.

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MMP 9 MEDIATES ANGIOPOIETIN-1 RECRUITMENT OF ENDOTHELIAL PROGENITOR CELLS TO DIABETIC WOUNDS

SG Keswani, JK Parvadia, ED Kozin, A Malik, PW Zoltick, A Radu, D Alaee, AB Katz, and TM Crombleholme
The Children's Hospital of Philadelphia, Philadelphia, PA 19104

Introduction: Adenoviral gene transfer of Angiopoietin-1 (AdAng1) recruits endothelial progenitor cells (EPCs) and improves diabetic wound healing. A suggested mechanism for EPC mobilization from the bone marrow (BM) is mediated through MMP-9 and stem cell factor (SCF). We hypothesize that Ang-1 recruits EPCs to diabetic wounds via an MMP-9 dependent mechanism.
Methods: Lethally irradiated mice were reconstituted with BM from transgenic TIE-2/LacZ mice. After engraftment, diabetes was induced with steptozotocin. 8 mm wounds were created in BM transplanted (BMT) ($n = 12$) or MMP-9 knockout (KO) ($n = 12$) mice and treated with 1×10^8 PFU of AdAng1, AdGFP or PBS. At 7 days wounds were analyzed for epithelial gap, vessel density, and EPCs. Serum levels of VEGF, proMMP9 and SCF were assessed. Data are expressed as mean ± SEM
Results: In diabetic BMT wounds, AdAng1 results in improved reepithelialization (Ang1 2.3 ± .2 mm; GFP 3.9 ± .2; PBS 4.0 ± .1 p < .0001) neovascularization (Ang1 6.8 ± .3Caps/Hpf; GFP 3.0 ± .4; PBS 2.9 ± .3 p < .0001) and EPC recruitment (Ang1 5.3 ± .4 EPCs/Hpf; GFP 2.1 ± .3; PBS 2.2 ± .3 p < .0001). AdAng1 treatment results in increased levels of proMMP-9 (Ang1 9.7 ± .8 ng/ml; GFP 6.3 ± .9; PBS 6.4 ± .4 p < .01) and SCF (Ang1 265 ± 28 pg/ml; GFP 119 ± 16; PBS159 ± 12 p < .001). In MMP9 KO mice, AdAng1 accelerates reepithelialization (Ang1 3.2 ± .1 mm; GFP 4.1 ± .2; PBS 3.8 ± .2; p<.) but has no significant effect on neovascularization (Ang1 4.1 ± .5 Caps/HPF; GFP 3.9 ± .4; PBS 3.9 ± .6), EPC recruitment (Ang1 2 ± .3 EPC/Hpf; GFP 1.5 ± .3; PBS 1.6 ± .2) or SCF levels (Ang1 83 ± 5 pg/ml; GFP 83 ± 2; PBS 88 ± 6).
Conclusions: The effects of Ang-1 on EPC recruitment and neovascularization are dependent on MMP-9. Our data support the hypothesis that MMP-9 enables SCF to permit mobilization of EPCs.

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SYNERGISTIC EFFECT OF TGF BETA 1 AND HIF-1A ON THE EXPRESSION OF A HYPOXIA-RESPONSIVE ELEMENT-CONTAINING PROMOTER

Nakshatra K. Roy (n-roy@northwestern.edu), Alan C. Sull, Thomas A. Mustoe

Wound Healing Research Laboratory, Department of Surgery, Northwestern University, Chicago, IL 60611, USA.

Accumulating data strongly suggests that under hypoxic conditions the adaptive physiological response of the cells involves co-operation between oxygen sensing and growth factor signals that cause HIF-1 mediated gene expression. The HIF-1 has been identified as a central and critical molecule in oxygen sensing and possibly a master switch. Of the many cytokines that have been shown to modulate collagen production, TGFβ1 appears to be crucial as it can sustain stimulation of collagen production as well as autoinduction of its own synthesis. Several genes that are known to be hypoxia inducible are also up-regulated by TGFβs, and the promoter of TGFβ3, a member of the family, has a hypoxia-response element (HRE). Recent reports indicate that HIF-1α physically interacts with Smad3 and that the TGFβ and hypoxia signaling pathways synergize at the transcriptional level to regulate gene expression. To determine if this interaction upregulates gene expression through the HRE element, we have co-transfected cultured human dermal fibroblasts with the mammalian expression plasmid for HIF-1α (pCEP1-HIF-1α) with a reporter construct 5HRE-luc, containing a concatemer of five copies of HRE derived from human vascular endothelial growth factor (VEGF), a minimal cytomegalovirus (CMV) promoter and a reporter gene,luciferase.). When treated with 2 ng/ml of TGFβ1 protein, co- transfected hypoxic aged and young human dermal fibroblasts showed significant synergistic upregulation of the reporter gene expression. To further confirm the TGFβ-HIF-1α interaction we have created a TGFβ1 -RNAi construct by subcloning a 19-nucleotide sequence derived from rabbit in a mammalian expression vector (p-SUPER) that directs the synthesis of small interfering RNAs (siRNAs). . Hypoxic rabbit cultured dermal fibroblasts co-transfected with TGFβ1-RNAi and 5HRE-luc showed down-regulation of reporter gene expression.
As a prerequisite to understanding the biology of physiological and pathological ischemic tissue repair process it is important to delineate the molecular basis of regulation of collagen gene expression in fibroblasts. Each step in the TGFβ signaling cascade is a potential target for highly specific therapy and information about how hypoxic low-oxygen microenvironments affect the TGFβ cascade could be useful to develop novel therapeutic strategies that will involve agonist and antagonist agents that directly interfere with these steps.

We thank Drs. G. Semenza, Jons Hopkins Univ and J M.Brown, Stanford Univ, for their generous gifts of pCEP1-HIF1α and p5HRE-hCMVmp-luc respectively.

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TRANSFORMING GROWTH FACTOR-BETA LIGAND, RECEPTOR, AND SMAD EXPRESSION PATTERNS IN FETAL AND ADULT KERATINOCYTES

HP Lorenz, R Faudoa, MT Longaker, TM Krummel

Pediatric Surgical Research Program, Department of Surgery, Stanford University, Stanford, CA USA

Introduction: The transforming growth factor-beta (TGF-ß) cytokine family regulates cellular proliferation, differentiation, and migration with important function during both development and repair. Moreover, the TGF-ßs are growth inhibitory for keratinocytes, proliferative for fibroblasts, and generally profibrotic during repair. In order to better define the influence of keratinocyte TGF-ß during these processes, we examined the TGF-ß isoform, receptor, and signal messenger Smad expression in fetal and postnatal keratinocytes.
Methods: Sprague-Dawley rat keratinocytes were isolated in primary culture from fetal at E15 and E17 (term = E22), newborn, and 6 week old adults. Open fetal rat wounds heal without scar at E17 gestation. Quantitative-polymerase chain reaction was performed for TGF-ß1, -ß2, and -ß3 ligand; TGF-beta receptor1 and -receptor2; Smad3, Smad4, and Smad7 expression. All cells were passage 2 or 3.
Results: TGF-ß1 expression did not change appreciably from E15 to adult age in keratinocytes. TGF-ß2 and -ß3 expression increased (8 fold and 2 fold respectively) from E17 to newborn ages. Overall, the expression of TGF-ß1 was 5–8 fold greater compared to -ß2 and -ß3 in both fetal and adult keratinocytes. TGF-ß receptor1 expression increased 2 fold whereas -receptor2 expression showed little change from E17 to adult age. Smad3 expression increased over 50 fold, with Smads 4 and 7 showing a smaller increase from E17 to adult age.
Conclusions: The TGF-beta system has differential expression in fetal compared to postnatal keratinocytes, which suggests function during skin differentiation. TGF-ß1 expression is relatively greater than -ß2 and -ß3 in both fetal and adult keratinocytes. However, increases in isoforms -ß2 and -ß3, receptor1 and the Smads occurs at ages associated with scarring, implying increased function of the pro-fibrotic TGF-beta response during ages associated with scarring.

Funding: NIH GM-041343–14

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CM-1 (CYTOMODULIN), A SYNTHETIC PEPTIDE, *PROMOTES* COLLAGEN TRANSCRIPTION AND WOUND HEALING IN BIOLUMINESCENT MICE

Jayasri Dasgupta*, Rajendra Bhatnagar and Jeffrey M Davidson*.
*Department of Pathology, Vanderbilt University and †Research Service, VA Med Ctr., Nashville, TN, University of California San Francisco, and MediScient, Inc., San Mateo, CA **(jayasri.dasgupta@vanderbilt.edu)**

Cytomodulin (CM-1) is a synthetic heptapeptide. Human dermal fibroblasts cultured *in the presence of CM-1* showed increased expression of transforming growth factor–ß (TGF-ß) and collagen I and decreased expression of MMP-1. TGF-β is involved in numerous vital processes in wound healing, and the rate of wound repair is influenced by its overexpression at the site of injury. To establish the effect of CM-1 in wound healing, we administered several concentrations of CM-1 to incisional wounds and to unwounded skin of collagen-luciferase transgenic mice. Luciferase bioluminescence served as a quantitative reporter of CO1A2 transcription. Imaging was performed to measure the luciferase activity on these mice at different time points after administration of 1, 10 and 100 ug CM-1. The highest amount (100 μg) showed the highest level of collagen expression both in unwounded and wounded skin. In another experiment, we used a scrambled peptide as a control and administered the peptides both topically and by injection. CM-1 was effective in stimulating luciferase expression both by topical and intradermal administration. To determine the effect of CM-1 on wound strength, we measured the tensile strength in incisional wounds of wild type C57B6 mice at 10 d. Histological analysis was also performed to assess the collagen content and organization of the tissue. The data showed a statistically significant increase in tensile strength and collagen content at 10 and 100 μg doses. These results illustrate the utility of CM-1 as a novel, low-molecular weight vulnerary agent.

Supported by NIH grant AG06528 and the Department of Veterans Affairs.

POSTERS

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EFFECTS OF FAT AUTOGRAFTING ON PORCINE SKIN WOUND HEALING AFTER INJURY

Lijun Fang, Xiaobing Fu, Yuxin Wang, Tongzhu Sun, Biao Cheng.

Wound Healing and Cell Biology Laboratory, Burn institute, 304th Hospital, Trauma Center of Postgraduate Medical College, Beijing 100037, P.R.China (*fuxb@cgw.net.cn*; *fuxiaobing@vip.sina.com*)

It has been evidenced that the fat may have a potential to secret some growth factors or be a source of stem cells. So, we explore the effects of fat on healing of porcine skin wounds so as to provide a new method for clinical skin wound repair after injury.
Forty-eight full-thickness skin wounds were produced on both sides of the back in 6 male minipigs (8 wounds in each animal). Then these wounds were randomly divided into 4 groups, which were saline control group, fat autografting group, basic fibroblast growth factor (bFGF) treatment group and epidermal growth factor (EGF) treatment group. At day 3, 7, 14 and 21 after wounding, the area and the volume of wounds were measured and the histological examination was performed to evaluate the velocity and quality of wounds healing in different groups.
At day 3 and 7, the amount of granulation tissues and vessel density in fat treatment group were significantly more than that in other groups. Wound areas and volume in fat treatment wounds were markedly decreased in compared with those in other groups ($P < 0.01$). Regenerated epidermis in fat treatment group was thicker than that in other groups.
These results confirmed that the wound healing velocity and quality in wounds treated with fat autografting were enhanced. It indicated that fat has a potential to accelerate velocity and improve the quality of wound healing after skin injury.

This study was supported in part by the National Natural Science Foundation of China (30170966, 30230370), National Basic Science and Development Programme (973 Programme, G1999054204)

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BONE MARROW MESENCHYMAL STEM CELLS AS A POTENTIAL SOURCE TO REPAIR THE INJURY SKIN

Xiaobing Fu,[1, 2] Lijun Fang,[1, 2] Xiaokun Li,[1, 3] Biao Cheng,[1] Zhiyong Sheng[1]

1 Wound Healing and Cell Biology Laboratory, Burn Institute, 304th Hospital, Trauma Center of Postgraduate Medical College, Beijing 100037, P.R.China (*fuxb@cgw.net.cn*; *fuxiao bing@vip.sina.com*)
2 Department of Stomatology of the Second Clinical College, China Medical University, ShenYang 110003
3 Pharmaceutical Division, Jinan University, Guangzhou 510632

Adult stem cells are found in various tissues and organs and have potential to differentiate into different cell lineages, including bone, cartilage, fat, tendon, muscle, epithelial cells of the skin and gastrointestinal tract, etc. Here, we report that the expended and purified bone marrow MSCs might take on phenotypes and characteristics of vascular endothelial cells or epidermal cells after cultured and induced with different lineage-specific culture conditions in vitro. Also, in vivo grafting experiments confirmed that those labeled MSCs could get the phenotypes of vascular endothelial cells in granulation tissue and sebaceous duct cells and epidermal cells in regenerated skin, which imply that these grafted MSCs might have transdifferentiated into vascular endothelial cells and sebaceous duct cells and epidermal cells. Our results indicate that locally delivered bone marrow MSCs may generate *de novo* intact skin and get perfect skin regeneration after full thickness injury.

This study was supported in part by the National Natural Science Foundation of China (30170966, 30230370), National Basic Science and Development Programme (973 Programme, G1999054204)

086

SKIN WOUND HEALING IN RED DUROC X YORKSHIRE F1 PIGS: EFFECT OF WOUND DEPTH ON GENE EXPRESSION

CL Gallant, ME Olson, DA Hart

University of Calgary, Calgary, Alberta, CANADA, T2N 4N1

Previous investigations have demonstrated that cutaneous healing in red Duroc pigs (RD) differs from healing in Yorkshire pigs (Y). Specifically, RD animals heal with the formation of hypercontracted, hyperpigmented scars, features which are not observed in Y animals. Further, this abnormal scarring may be genetically mediated, as demonstrated by the partial transmission of this phenotype to RD × Y offspring (F1). To further determine the degree of transmission of this phenotype, this study examined the effect of wound depth upon the healing response of F1 animals. Ten full thickness (FT) and ten deep dermal (DD, 1.8 mm) skin wounds (2 cm × 2 cm) were created on the backs of juvenile female F1 animals (N = 8) using previously published procedures. Biopsies (4 mm) were taken from FT and DD wounds on days 14, 28, 42, 56, and 70. Samples were used for histology and molecular analysis using RT-PCR. There were no detectable differences between the FT and DD wounds on the gross or histologic level. However on a molecular level, there were numerous differences between the FT and DD wounds. Several genes investigated demonstrated increased expression levels in the DD wounds as compared to the FT wounds. Further, DD wounds demonstrated a unique pattern of expression for several other genes that differed from that observed in either the F1 FT wounds or in any RD or Y wounds. Given that previous investigations have shown that FT and DD wounds are nearly identical within each of the RD and Y strains, this represents a novel finding. These results indicate that the healing phenotype of the F1 animals may be regulated both by genetics and by the wound size/depth, suggesting that human wound healing may be similarly influenced by genetics, wound depth and potentially, environmental factors.

Funding provided by NSERC, CIHR.

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NORMAL OR PATHOLOGICAL WOUND HEALING: APOPTOSIS SENSITIVITY OF MYOFIBROBLASTS

V. Moulin, S. Larochelle, C. Langlois, I. Thibault, C. A. Lopez-Valle, M. Roy

LOEX, Saint-Sacrement Hospital/CHA, surgery department, Laval University, Québec, Canada.

During wound healing, myofibroblasts play a major role in wound contraction and matrix formation. At the end of healing, myofibroblasts disappear via apoptotic pathways. Hypertrophic scars are a fibroproliferative disorder that leads to considerable morbidity. Although no evidence exists, it has been postulated that a defect in myofibroblast apoptosis could be responsible for the pathological scar formation. We have isolated and cultured human normal wound (Wmyo) and hypertrophic scar (Hmyo) myofibroblasts and compared their basal apoptotic rates and their sensitivity to serum starvation and Fas antibody-induced apoptosis to that obtained for dermal fibroblasts (Fb). A higher rate of apoptosis as evidenced by morphological criteria and a propidium iodide assay was observed for Wmyo in comparison to Fb and Hmyo. These results came along with a low level of the anti-apoptotic proteins Bcl-2 and $Bclx_L$ in Wmyo, whereas there was an increase in the level of the pro-apoptotic molecule Bax when compared to the results obtained for Fb and Hmyo. Hmyo showed a higher level of Bcl-2 compared to Fb but no difference in the Bax or $Bclx_L$ level. After serum starvation, Wmyo revealed an increased apoptotic rate whereas Hmyo and Fb did not show any difference. Anti-Fas treatment did not modify the levels of apoptosis but strongly increased the cell growth of Hmyo as compared to Wmyo. This is the first study presenting a broad vision of the apoptotic sensitivity of normal and pathological myofibroblasts. These results confirmed the hypothesis of defects in apoptosis and growth during pathological scar formation impeding myofibroblast disappearance at the end of healing.
Acknowledgements: This study was supported by the Canadian Institutes of Health Research (#MOP-42400) and "Fondation de l'Hôpital du Saint-Sacrement". S.L. and V.M. were recipients of scholarships from "Centre de recherche du CHA".

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TOPICAL SUBSTANCE P MODULATES INFLAMMATORY RESPONSES IN HEALING WOUNDS IN NITRIC OXIDE SYNTHASE KNOCKOUT MICE

P Muangman, RN Tamura, LA Muffley, F Frank Isik, NS Gibran

Introduction: Substance P (SP), a proinflammatory neuropeptide released by sensory nerves in response to cutaneous injury, increases nitric oxide (NO) production by target cells. SP upregulates three nitric oxide synthase (NOS) isoforms: neuronal (nNOS), inducible (iNOS) and endothelial (eNOS). Topical SP application improves wound healing kinetics in NOS KO mice. Since nitric oxide may be a key intermediary mediator for SP induced response to injury, this study was designed to determine whether SP promotes inflammatory cell density in early wounds of NOS KO mice.
Methods: Mice of each NOS null strain and appropriate background controls (C57BL/6J – i/eNOS control;B6129SF2/J – nNOS control) were anesthetized with intraperitoneal ketamine and xylazine. After shaving, full-thickness 1.5 × 1.5 sq. cm. dorsal excisional wounds were covered with a semi-occlusive dressing. Mice were randomly assigned to daily topical infusion of 10^{-7} M SP or NS onto wound beds. Wounds were harvested at day 3 and 7 (3 mice per group). Cells were isolated by sequential digestion in dispase and hyaluronidase. Cell counts were performed to determine total cell density. Flow cytometry was performed after staining cells with FITC – labeled antibodies against F4/80 (macrophages), CD45 (pan leukocytes), CD31 (endothelial cells) or CD11c (dendritic cells).
Results: Total cell number was higher at day 7 post-wounding than at day 3 ($P < 0.05$) in both control and NOS null mice. Macrophage, pan-leukocyte and dendritic cell density was greater in the SP-treated wounds than in NS-treated wounds at both day 3 and 7 post-wounding ($P < 0.05$) in all NOS KO mice. Whereas SP increased endothelial cell number in nNOS and iNOS mice, SP had no effect on endothelial cell number in the eNOS KO mice ($P > 0.05$). SP did not affect cell density in background mice at either time point ($P > 0.05$).
Conclusion: SP upregulated inflammatory cell numbers in all NOS null mice suggesting that either SP activates NOS isoforms that are not reduced by targeted deletion or that SP induces inflammatory cell migration by NO-independent pathways. The lack of SP effect on endothelial cell number in eNOS KO wounds suggests that eNOS may mediate SP-induced wound angiogenesis.

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TENSILE STRENGTH DEFICIENCY IN SKIN AND WOUNDS OF SUBSTANCE P RECEPTOR (NK1R) KNOCKOUT MICE

C D Carlisle[1], J DasGupta[1], J Ansel[2], J M Davidson[1,3]
[1]Department of Pathology, Vanderbilt University, [3]Research Service, Department of Veterans Affairs Medical Center, Nashville, TN[2]; Department of Dermatology, Northwestern University, Chicago, IL

Diabetic neuropathy leads to reduced innervation that may in turn diminish the availability of tachykinins such as Substance P (SP). In diabetic mice, exogenous SP has positive effects on wound closure. The role of SP was tested by inactivating the murine SP receptor gene (neurokinin-1 receptor, NK1R). Age-matched, $NK1R^{-/-}$ mice and C57/BL6 control mice were each given 2 full thickness 6 mm excisional wounds on their dorsal surface and creation of two dead space wounds by the insertion of two 1×3 mm PVA sponge disks under the skin through a 1.5 cm long ventral midline incision. Digital images for wound closure analysis were collected until day 10 when tissues were harvested for biochemical, histological and tensiometric analysis.

There was a striking reduction (2.5 fold, $p < 0.05$) in the tensile strength of intact, unwounded skin of the $NK1R^{-/-}$ mice, and tensile strength of 10 day incisions was likewise reduced (1.8 fold, $p < 0.0126$). Wound closure rates were also reduced in the knockout mice, but not to a significant level. Sponge granulation tissue had equivalent protein and DNA content at 10 day post-implantation while collagen content was diminished. Histological examination of excisions, normal skin and sponges showed no readily observable difference in thickness or organization however $NK1R^{-/-}$ mice exhibited visibly less collagen in excisional wounds. The principal dermal effect seen in this gene deletion appears to be the accumulation and organization of connective tissue.

Our preliminary results with a non-diabetic $NK1R^{-/-}$ mouse do not indicate a marked difference in reëpithelization, but instead a significant decrease in biomechanics. The parallel diminution of intact skin and wound strength suggests an intrinsic defect in collagen fiber maturation that may be reflected in ultrastructural organization or covalent crosslinking.

Acknowledgements: This research was funded by the Department of Veterans Affairs, and NIA grant AG-06528. Our thanks to the Vanderbilt University SDRC Phenotype Core for Animal Surgical Services and Mouse Pathology Core.

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EFFECT OF CYTOKINES AND HEAT SHOCK ON HUMAN β-DEFENSIN-2 LEVELS IN CULTURED KERATINOCYTES

Satyanarayan Bhat[1], Roger J. Bick[2], Brian J. Poindexter[2], Salil Gulati[1], Maximilian Buja[2] and Stephen M. Milner[1]
[1]The Institute for Plastic and Reconstructive Surgery, Southern Illinois University School of Medicine, Springfield, Illinois 62794-9653[2] The Department of Pathology and Laboratory Medicine, University of Texas Medical School at Houston, Houston, Texas 77030

Burns have been associated with high levels of circulating pro-inflammatory cytokines which promote systemic inflammatory response syndrome (SIRS), immunosuppression and sepsis for which no effective treatment is currently available. Defensins, a family of cationic naturally occurring antimicrobial peptides are considered important components of the innate immune system and enhance adaptive immunity. We have examined the effects of pro-inflammatory cytokines, interleukin 1β- (IL-1β), gamma-interferon (IFNγ) and tumor necrosis factor-α (TNFα) on human β-defensin-2 (HBD-2) levels in cultured keratinocytes. We have also examined the effects of heat shock at 42 °C. Our results demonstrate that only TNFα shows significant induction of HBD-2 but this induction was not sustained in the long term. In addition, endogenous levels of defensin were significantly reduced by exposure to heat shock. The keratinocytes also responded to IL-1β by becoming hypertrophic. These results indicate that stress-related, pro-inflammatory cytokines can induce keratinocytes to synthesize HBD-2, while heat shock appears to reduce its production. Understanding the roles cytokines play in the continued problems associated with severe wounding, and in particular, major burn injury, is important. This study provides further insight into the role of natural anti-microbial peptides under conditions of stress.

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EXPRESSION OF HUMAN β-DEFENSIN-2 IN THE SKIN APPENDAGES OF BURN WOUNDS

Salil Gulati[1], Roger J Bick[2], Satyanarayan Bhat[1], Brian J. Pondexter[2], Maximilian Buja[2] and Stephen M Milner[1].
[1]Institute for Plastic Surgery, Southern Illinois School of Medicine, Springfield, Illinois 62794 [2]Department of Pathology and Laboratory Medicine, University of Texas Medical School at Houston, Texas Health Science Center, Houston, Texas 77030

Major burn wounds are often complicated by sepsis which currently accounts for over 54% of deaths in these patients. Defensins, a family of cationic naturally occurring antimicrobial peptides are considered important components of the innate immune system as they play a major role in body's defence by inhibiting several bacteria, fungi and enveloped viruses. They also chemoattract immature dendritic cells and T and B-lymphocytes, neutrophils and macrophages and act as an adjuvant and enhance adaptive immunity. Our prior studies using RT-PCR suggest decreased expression of human β defensin-2 (HBD-2) in burn wounds. Furthermore we demonstrated remnants of HBD-2 in the dermis. Our current study, using fluorescence deconvolution microscopy, has clarified that HBD-2 is present in the surviving hair follicles. The peptide was also located in the eccrine and apocrine sweat gland acini but absent in the sweat ducts, in representative skin samples of deep partial and full thickness burns. HBD-2 was also notably absent from the vascular endothelium and fat cells. 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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ARP2/3 MESSENGER RNA LOCALIZATION AND FIBROBLASTS MIGRATION IN CULTURE AND IN RAT HEALING WOUNDS

Gang Liu, Lisa A. Mingle, Nataly N. Okuhama, Livingston Van De Water and Robert Harmon, Center for Cell Biology and Cancer Research, Albany Medical College, NY

Directed cell migration plays an important role in wound healing and many other cellular processes. To move directionally, a cell must become asymmetric and establish a dominant protrusion in which localized actin polymerization is a hallmark. We demonstrate that Arp2/3 protein complex, an actin polymerization nucleator consisting of seven protein subunits, is localized to the leading protrusions of cells in culture and in rat healing wounds. The localization of the Arp2/3 protein complex is consistent with its function to promote protrusion and migration. However, the mechanism that restricts the localization of this complex in migrating cells remains unknown. We demonstrate that messenger RNAs (mRNAs) for all the seven subunits of the Arp2/3 complex are localized at the leading protrusions of cells in culture and in wounds. This is the first evidence that mRNAs encoding a protein complex are co-localized to a common site of function. Interestingly, mRNA for Dia-I, a formin protein and another actin nucleator for actin bundles (such as stress fibers), is not localized. Such differential localization of mRNAs for the two actin nucleators suggests that these two functions are sequestered by mRNA sorting. We also demonstrate that the Arp2/3 mRNA localization is dependent on both the actin and microtubule cytoskeletons because disrupting either system abolishes Arp2/3 mRNA localization. The expression and localization of Arp2/3 mRNAs are dependent on serum, indicating Arp2/3 mRNA expression and sorting are consequences of a cellular response to the extracellular environment. Current work focuses on identification of the localization signal sequences and the physiological significance of Arp2/3 mRNA sorting in directed fibroblasts migration.

Supported by AMC start up fund (GL) and NIH/GM56442-07 (LVDW).

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PROSTAGLANDIN E2 INHIBITS FIBROBLAST MIGRATION: IMPLICATIONS FOR WOUND HEALING

V.C. Sandulache, H.S. Li-Korotky, J.E. Dohar and P.A. Hebda
(Children's Hospital of Pittsburgh, Pittsburgh PA)

Background: Wound healing is a complex process involving multiple cell types, extracellular matrix components and soluble mediators. Prostaglandin E2 is an important component of the inflammatory response to injury. PGE2 can regulate the fibroblast response to injury via the EP receptor family. Here, we examine PGE2 regulation of fibroblast migration. Our analysis extends to fibroblasts representing a spectrum of wound healing phenotypes.
Hypothesis: Prostaglandin E2 mediated inhibition of fibroblast migration is conserved across multiple fibroblast phenotypes.
Methods: Primary cultures of human fetal, adult and keloid fibroblasts were used. Analysis of the EP receptor profile for each fibroblast phenotype was conducted using real-time PCR, Western blot and immunohistochemistry. Fibroblast migration was quantified using a well established in vitro scratch assay.
Results: Prostaglandin E2, via EP2/EP4 receptors, inhibits fibroblast migration in all fibroblast phenotypes. Fetal fibroblasts retain a more robust migratory phenotype when compared to normal adult and keloid fibroblasts. Normal adult fibroblasts exhibit a dramatic destabilization of the actin cytoskeleton which accompanies PGE2 inhibition of cell migration. This effect was not observed in fetal or keloid fibroblasts.
Conclusions: Fibroblast activity in the wound bed can be altered by inflammatory mediators. The effects of prostaglandin E2 appear to be partially conserved across various fibroblast phenotypes. Variability in the response of these cells, however, indicates that fibroblasts derived from fetal tissue may retain intrinsic altered response mechanisms to endogenous inflammatory mediators.

We thank Children's Hospital of Pittsburgh for financial support.

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FIBROBLAST TRANSMIGRATION FROM COLLAGEN INTO FIBRIN/ FIBRONECTIN GELS REQUIRES SYNDECAN-4 PROTEOGLYCAN

Fubao Lin*, Jianqiang An*, Xiangdong Ren#, Greiling Doris#, and Richard A.F. Clark*#
Department of Dermatology#, Medicine and Biomedical Engineering*, School of Medicine and College of Engineering and applied Science, SUNY at Stony Brook, Stony Brook, NY 11794-8165

Fibroblast migration from the peri-wound collagenous stroma into the fibrin-laden wound is critical for granulation tissue formation and subsequent healing. Previously we found that fibroblast transmigration from a collagen matrix into a fibrin matrix required the presence of fibronectin (FN). Several cell surface receptors, namely integrins $\alpha4\beta1$, $\alpha5\beta1$ and $\alpha v\beta3$ were also required for this invasive migration. Here we demonstrated that syndecan-4, a transmembrane heparan sulfate proteoglycan, known to bind FN at the highly cationic HepII domain is also required for fibroblast invasive migration of a fibrin/FN gel. This conclusion was based on fibroblast migration using two independent means of disrupting syndecan-4: heparinase degradation of heparan sulfate glycosaminoglycans or suppression of syndecan-4 core protein with antisense oligodeoxynucleotides. Isolated syndecan-4 from these fibroblasts bound the Hep II recombinant constructs FN III12–15v > FN III12–15 > FN III12–14 but did not bind the alternatively spliced IIICs (V) domain. Furthermore, we found that platelet-derived growth factor (PDGF), which is required to stimulate fibroblast migration, markedly increased cell levels of syndecan-4 core protein in a time and dose dependent fashion. PDGF also induced up-regulation of syndecan-4 at transcriptional level as determined by RT-PCR. These results demonstrate that syndecan-4 is essential for fibroblast invasive migration into fibrin clot and that PDGF, the stimulus for migration, induces increased syndecan-4 core protein expression.

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HSP70 IS PROINFLAMMATORY *IN VIVO*. IMPLICATIONS FOR CHRONIC WOUND HEALING.

Perdrizet**, G, Enderle, J. Rewinski, M**, R. Kumar, G. Enderle, M. Berman^x and Hightower*, L.
Dept of Biomedical Engineering, *Molecular and Cell Biology, University of Connecticut, Storrs, and ^xDepartment of Pathology and **Center for Wound Healing and Hyperbaric Medicine, University of Connecticut Health Center and Hartford Hospital, Hartford, CT.

Abstract: Failure to downregulate inflammatory responses is often used to explain why chronic wounds fail to heal. The beneficial effects of surgical debridement are thought to be in part due to the transformation of a chronic wound to an acute wound, thereby allowing the inflammatory phase of acute wound healing to be reactivated. We tested the hypothesis that subcutaneous injection of the inducible isoform of the 70 kDa mammalian heat shock protein (iHSP70) will cause acute inflammatory swelling in an *in vivo* murine model. Comparisons between groups were performed using the Student's t-test and significance was accepted when $p < 0.05$. Subcutaneous injection of stress proteins iHSP70 was associated with significantly increased acute swelling in comparison to injections of saline, autologous serum (AS) or denatured iHSP70, $*p < 0.05$, see figure below. Histological analysis demonstrated increased inflammatory cell infiltrate in those tissues receiving iHSP70. Stress proteins, within the acute wound environment, are proinflammatory and may set the stage for persistent inflammation within the chronic wound bed. Stress proteins may be future targets for therapeutic manipulation wherever acute inflammation fails to be downregulated.

Exp. Group	N	Swelling(mm)-24 hrs	Swelling(mm)- 48 hrs	p vs iHSP70
Saline	9	0.5 ± 0.25	0.6 ± 0.15	0.001
Serum	8	0.73 ± 0.18	0.72 ± 0.17	0.001
iHSP70	11	1.67 ± 0.36	1.55 ± 0.22	ns
Denatured iHSP70	10	1.48 ± 0.41	1.25 ± 0.24	0.05

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BIOCHEMICAL ANALYSIS OF CHRONIC WOUNDS TREATED WITH THE V.A.C.® SYSTEM

J. K. Stechmiller[1] B. B. Childress[1], T. D. Blalock[2], D. V. Kilpadi[3], R. W. Nappo[4], G. A. Chin[4], D. W. Mozingo[4], & G. S. Schultz[2]
[1]Univ of FL College of Nursing, [2]Institute of Wound Research, Dept of Obstetrics and Gynecology, Univ of FL, Gainesville, FL; [3]KCI, San Antonio, TX; [4]Dept of Surgery, Univ of FL, Gainesville, FL

Introduction: Proinflammatory cytokines TNF-α, IL-1β, and matrix metalloproteinases (MMPs) are elevated in chronic wounds and their levels decrease during healing. TNF-α and IL-1β stimulate the production of MMPs which cause degradation of growth factors. Wound fluid from the V.A.C.® is of interest when investigating the mechanisms by which V.A.C.® therapy aids in wound healing. We hypothesize that the clinical improvement in healing of chronic wounds by the V.A.C.® system occurs by a shift from a chronic cytotoxic environment to a more conducive acute healing wound environment. This study aims at understanding the molecular and mechanistic consequences of V.A.C.® therapy on chronic wound healing.
Methods: Wound fluid collected from two patients with Stage IV pressure ulcers at baseline, 24hours, 72hours, and 7 days following V.A.C.® therapy. Concentrations of TNF-α, IL-1β, MMP-2, -3, -9, TIMP-2, and total protein were determined using ELISA.
Results: TNF-α levels in wound fluids decreased from baseline levels in two patients following 3 days of treatment. Similarly, levels of MMP-3, MMP-9 and TIMP-2 decreased from baseline following 3 days of treatment. There were no consistent trends in IL-1β & MMP-2.
Conclusion: The preliminary results from this study suggest that improved wound healing with V.A.C.® therapy is related to decreased levels of proinflammatory cytokines, MMPs and TIMP-2 in wound fluid.

This study was funded by Kinetics Concepts, Inc., San Antonio, TX.

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COMPARATIVE ANALYSIS OF HLA-G EXPRESSION BY KERATINOCYTES DERIVED FROM PEMPHIGUS VULGARIS PATIENTS VS. NORMAL INDIVIDUALS

F. Yari[1], A. Zavaran Hosseini[2], M. Nemat-Gorgani[3], Parvin Mansouri[4], and M.R. Khorramizadeh[5]

1- PhD student of Immunology, Iran Blood Transfusion Organization, Tehran, Iran.
2- Associate Professor, Dept. of Immunology, School of Medical sciences, Tarbiat Modarres University, Tehran, Iran.
3- Associate Professor, Institute of Biochemistry and Biophysics, University of Tehran, Iran.
4- Professor, Dept. of Dermatology, Tehran University of Medical Sciences, Iran.
5- (Correspondence) Assistant Professor, Div. of Biotechnology, Dept. of Pathobiology, School of Public Health, Tehran University of Medical Sciences, P.O.Box 14155-6446, Tehran, Iran, Tel: +98-21-6462268 Fax: +98-21-6462267 E-mail: khorami@yahoo.com

Background: Human leukocyte antigen-G (HLA-G), is a non-classical HLA class I molecule. Over the past few years, HLA-G has been considered as a tolerogenic antigen and a tissue protective molecule in inflammatory diseases.
Objectives: This study was performed to analyze HLA-G expression in pemphigus vulgaris (PV) patients and to assess its possible anti-inflammatory role in skin disorders.
Methods: With observing ethical issues, skin biopsies from 4 normal subjects and 3 PV patients were prepared. The epidermal layer of these biopsies was separated using Dispase enzyme treatment. After trypsinization, single cell suspensions of keratinocytes were cultured in MCDB-153 serum-free medium. Three weeks supernatants were collected and applied for a sandwich ELISA to detect total soluble isoforms of HLA-G antigen. Two species of antibodies used for ELISA were made in our laboratory, i.e. anti-HLA-G polyclonal and 5E6H7 monoclonal. A reverse transcriptase polymerase chain reaction (RT-PCR) was developed for pan HLA-G transcript to assess the regulation level of HLA-G expression.
Results: The data on ELISA experiments showed significant HLA-G expression by PV kertainocytes ($p < 0.001$). No traces of HLA-G production were seen in normal cells. RT-PCR revealed, however, variation in HLA-G mRNA expression. These findings indicate that regulation of HLA-G gene might occur both at transcriptional and translational levels.
Conclusions: Collectively, our data show the presence, and hence, advocate the possible protective contribution of HLA-G isoforms in skin inflammatory processes.

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SMOKING, ATHEROSCLEROSIS, AND MCP-1 EXPRESSION

Lina Wong[1, 2], Hongwei Yuan[1], Manuela Martins-Green[1]

[1]**Department of Cell Biology and Neuroscience**, [2]**Graduate Group of Biomedical Sciences, University of California, Riverside CA 92521**
**Both authors contributed equally to this work.*

The inflammatory events of atherosclerosis involve monocytes, endothelial cells, smooth muscle cells, and several cytokines, including chemokines. Atherosclerosis begins with recruitment of circulating monocytes to sites of blood vessel injury. In the vessel wall, these cells are activated to become macrophages that scavenge toxic material such as oxidized LDL and transition into "foam cells" which will eventually lead to plaque formation. Tobacco smoke is known to exacerbate cardiovascular disease but the mechanisms by which this occurs are not known. The chemokine MCP-1 is involved in recruiting monocytes to the injured blood vessel wall and is commonly found in atherosclerotic plaques. Our data show that both "first-hand" and "second-hand" cigarette smoke stimulate MCP-1 production in cultured primary human aortic endothelial cells (EC), suggesting that cigarette smoke may contribute to plaque formation by stimulating aortic EC to produce MCP-1 in blood vessels after smoke injury. To test whether MCP-1 links smoking and cardiovascular disease, we are studying the expression of this molecule in mice that are either transgenic for human ApoB or are null for MCP-1 or its receptor, CCR2, by exposing the mice to smoke in a way that closely resembles human smoking. Blood samples from the mice were tested for carboxyhemoglobin, triglycerides, LDL and HDL, and MCP-1 at successive weeks after initiation of smoking and the aortas isolated to analyze for plaque size and MCP-1 expression in the tissue. The carboxyhemoglobin test shows that the mice are exposed to smoke. Plasma MCP-1 and HDL levels are reduced in the smoking groups, whereas LDL, triglyceride, and total cholesterol remain unchanged. On one hand, the decrease in circulating HDL levels suggests that the reason smokers develop atherosclerosis may be because the rate of cholesterol clearance is slowed down. On the other hand, a decrease in circulating MCP-1 level may reflect that this chemokine accumulates in blood vessel walls, conferring stronger ability for attraction of monocytes.

This work is supported by TRDRP and Phillip Morris.

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"SMART" TARGETING OF ANTI-SCARRING AGENTS IN AN IN VITRO MODEL OF RETINAL WOUND HEALING

Authors:F E Dhawahir, C Sheridan, D Kent, D Wong, I Grierson, S Roberts

Unit of Ophthalmology, Department of Medicine and Department of Chemistry, University of Liverpool, UK

Scarring causes the irreversible loss of vision seen in macular degeneration and retinal detachment. Treatment of both conditions is ineffective. Cells within the retina, retinal pigment epithelial cells (RPE), are pivotal in the formation of these diseases. Modifying the behavior of these cells may help in controlling the scarring process. We have shown that RPE involved in scarring, unlike normal RPE, express a cell surface sugar called the Thomsen Friedenreich (TF) antigen. Binding by a naturally occurring substance from the common mushroom, called a lectin, can inhibit key RPE functions that are crucial to scar formation. The present study investigates the potential of conjugating this lectin and/or a monoclonal antibody to the TF antigen with known anti-proliferative agents (e.g. Mitomycin) in order to evaluate if the anti-scarring effects of the agents are additive.

Type acknowledgements here

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AN OPEN, RANDOMISED, EXPLORATIVE, PHASE II, INVESTIGATION COMPARING ENAMEL MATRIX DERIVATIVE PROTEINS (EMD) AND CONTROL IN PATIENTS WITH PRESSURE ULCER STAGE II

Dierick, AM. MD[1], Van De Looverbosch, Dirk MD[2], Heyman, Hilde RN[2], Van De Looverbosch, Dieter MS[3]
Klina-Hospital, Antwerp[1]; Extra Mural Department Geriatrics, Antwerp[2]; University of Gent[3]

Background: Enamel Matrix Derivative proteins (EMD) have been used as local adjunct to periodontal surgery in more than 600.000 patients. EMD is approved in Europe, USA and Japan as a medical device class III. There have been no negative reports concerning the safety of the EMD. This is the first reported controlled investigation in a series of ongoing investigations on EMD application in chronic wounds and burns.
Aim of the study: The investigation was designed as an open randomised investigation with EMD (30 mg/ml) or vehicle control that consists of propylene glycol alginate (PGA) only. Ten patients were planned to be included. Male or female patients were >18 years of age and had had pressure ulcer present for more than 1 month. Patients with controlled diabetes were also included in the study. Patients with sacral pressure ulcer were to be excluded. EMD or *vehicle control* was applied weekly for up to 8 weeks. All patients received a secondary dressing (Mepilex® Border). Ulcer status and area were measured at week 0, 2, 4, 6, and 8. The primary objective was to measure the wound area and the percentage of viable/non-viable tissue. The secondary objectives were to evaluate ulcer tissue in biopsies taken before application (baseline) and at last visit. Tissue morphology was evaluated on haematoxylin-eosin-stained sections and the presence of EMD, selected growth factors, proliferating cells and myofibroblasts in tissue sections stained with monoclonal antibodies.
Regulatory authority and Ethical committee in Belgium approved the investigation.
Results: At the time of the abstract submission, results are not final. The histology and immunohistochemical evaluations will be performed November/December 2003.
Acknowledgement: Mölnlycke Health Care AB, Sweden, sponsored the investigation.

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VAC THERAPY VERSUS CONVENTIONAL THERAPY IN ACUTE AND CHRONIC WOUNDS

M.C. Obdeijn[1], A. Braakenburg[2], R. Feitz[3], J.H.G. Klinkenbijl[2]
[1]University Hospital Groningen, [2]Rijnstate Hospital Arnhem, [3]University Hospital Rotterdam

Introduction: Vacuum therapy is a new concept in wound treatment. Animal studies have shown good results in chronic and acute wounds. In clinical practice we have used the system for many wounds with very promising results. However there are few randomized clinical trials that prove the efficacy of the system.
Patients and methods: In 2002 we have started a randomized prospective clinical trial to compare the results of Vacuum Assisted Closure versus our wound management protocol with mainly foam dressings and alginates. In this study we have included acute and chronic wounds. The wounds were assessed 3 times a week by 3 researchers (a medical doctor and 2 nurses). We looked at the wound healing, described in terms of color, smell, temperature and aspect of the wound. The size and depth of the wound was recorded and photographs were taken. Once a week we did a swab of the wound. Besides the effect on wound healing we also looked at the time that was needed for dressing changes, the benefits for the patient and the nurses and the costs of the therapy.
Results: At this moment we have treated 35 patients. The preliminary results will be presented at the meeting.
With this study as an example we will also illustrate some of the problems that can be encountered when designing a clinical trial in wound healing.

We acknowledge KCI medical for there contribution to this study.

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EFFECTS OF TYPE II COLLAGEN AND BASIC-FGF ON CARTILAGE WOUND HEALING IN A 3D DEFECT MODEL

C.W. Chen[1, 2], Y.H. Tsai[1], C. Zuo[2], W.F. Lai[1, 2]
[1]Taipei Medical University, Taipei, Taiwan, ROC
[2]McLean Hospital/Harvard Medical School, MA, USA

Cartilage preserves limited regenerative capacity and results in insufficient healing after joint injury. Previous researches showed that biocompatible materials and growth factors enable to facilitate cartilage repair; however, the mechanisms of cartilage regeneration are still not completely understood. The purpose of this study was to determine the effects of extracellular matrix and growth factor on chondrocyte migration and phenotype during cartilage regeneration. Neocartilage, 35 mm in diameter and 1 mm in thickness, was fabricated by using rabbit primary chondrocytes embedded in an *in vitro* three-dimensional collagen matrix. Five mm diameter defects were made. The effects of collagen matrices and basic-FGF on three-dimensional cartilage wound healing were determined. Results showed that both type I and type II collagen matrices (1 mg/ml) facilitated migration of chondrocytes from surrounding cartilage into the defect area in the presence of 10 ng/ml basic-FGF. The average of migration speed was 91.5micron/day for type I, and 88.1micron/day for type II collagen matrix in the period of 26 days. Incorporation with basic-FGF, chondrocytes increased approximately 1.5 times motility in the both collagen matrices. However, the cartilaginous phenotype was noted only when embedded in type II collagen matrix. The results indicate that type II collagen may trigger the redifferentiation during the migration of chondrocytes. Extracellular matrix such as type I and type II collagen, in addition to cytokines such as basic-FGF, play an important role in the process of cartilage wound healing in a three-dimensional defect model.

This work was supported by National Science Council, Taiwan, R.O.C. under project no. NSC 91-2314-B-038-033.

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AUTOLOGOUS THROMBIN: CONTINUING STUDIES

May S. Jacobson, Sherwin V. Kevy
Children's Hospital, Boston, MA USA
Harvard Medical School, Boston, MA USA

Background: As many as 500,000 patients are exposed to bovine thrombin (BT) annually. This is increasing with the use of autologous platelet gel (PG) in surgery and in the treatment of chronic wounds (diabetic ulcers). The development of anti-phospholipid antibodies after exposure to bovine thrombin is of concern. We compared the kinetics of growth factor release by autologous thrombin (AT) to BT.
Methods: Whole blood is incubated with 95% ethanol and then centrifuged in the Smart-PReP™2 system (Harvest Technologies, Plymouth, MA) simultaneously with the preparation of a platelet concentrate (PC). The supernatant containing thrombin activity is collected. Clotting studies were done. The kinetics of growth factor release was determined by collecting the supernatant from the PG at 2 hours and daily thereafter for 7 days. The PC's were prepared in two concentrations by adjusting the volumes to 10 mL and 7 mL. The activation of the PC was done at a 10:1 ratio (PC:BT) at 7 mL and 10 mL and 3:1 and 5:1 (PC:AT) at 7 mL.
Results: Following a 45 minute incubation period the mean clotting time of PC by AT at a 3:1 ratio was 14.3 sec and at a 5:1 ratio was 22 sec (n = 13). Thrombin activity remained stable for 6 hours. Baseline PDGF-AB levels with AT was 50 ng/mL with a maximum of 106 ng/mL. BT using a 10 mL PC had a baseline level of 100 ng/mL with no increase over time. TGF-B1 studies demonstrated an immediate release of 98 ng/mL with a maximum level of 168 ng/mL for AT compared to BT with an initial level of 107 ng/mL and a maximum of 152 ng/ml.
Conclusions: AT produces a sustained release of growth factors during a seven-day period that is similar to BT at a 10:1 ratio. AT can be prepared simultaneously with the preparation of a PC.

Harvest Technologies and Children's Hospital Transfusion Fund.

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EFFECT OF CONNECTIVE TISSUE GROWTH FACTOR ON PROTEIN KINASE EXPRESSION AND ACTIVITY IN HUMAN CORNEAL FIBROBLASTS

S.S. Radhakrishnan[1], T.D. Blalock[2], G.S. Schultz[1]
[1]Institute for Wound Research, University of Florida College of Medicine, Gainesville, FL USA, [2]Schepens Eye Research Institute, Harvard Medical School, Boston, MA USA

Purpose: To evaluate expression patterns of protein kinases and protein kinase activities in human corneal fibroblasts treated with connective tissue growth factor (CTGF).
Methods: Human corneal fibroblast cultures were grown to confluence and treated with CTGF for 0, 5, and 15 minutes. Cytoplasmic protein extracts were obtained; protein kinase expression and activity arrays were performed using Kinetworks™ analysis (screening for expression of 75 different protein kinases and 31 different phosphoproteins). Further studies using extended time courses of CTGF exposure in corneal fibroblasts (0, 1, 2, 3, 4, 5, 10, 15, 30, and 60 minutes) were performed using immunoblot analysis to detect expression of protein kinase A catalytic subunit (PKA-cat), and focal adhesion kinase (FAK). All results and were normalized by comparison to beta-actin.
Results: After 5 minutes of exposure to CTGF, levels of active proteins increased for 21 of the 75 kinases analyzed. Some notable protein kinases that were induced include: death-associated kinase 1, focal adhesion kinase (FAK), G-protein coupled receptor kinase 2, protein kinase A catalytic subunit (PKA-cat), protein kinase B alpha, and protein kinase C (ϵ, μ, and ζ, subunits). Extended time course analysis of PKA-cat and FAK showed statistically significant increases in expression following CTGF stimulation within 15 minutes.
Conclusion: CTGF increased the levels of active protein kinases in human corneal fibroblast cultures, including PKA-cat and FAK after 5 minutes of exposure. These results further our understanding of the signal transduction mechanism activated by CTGF in corneal fibroblasts. This suggests that CTGF mediates the effects of transforming growth factor beta (TGF-β) on protein kinase expression and phosphorylation of second messengers in processes such as cell proliferation and collagen synthesis.
Acknowledgments: Funding source: NEI 05587

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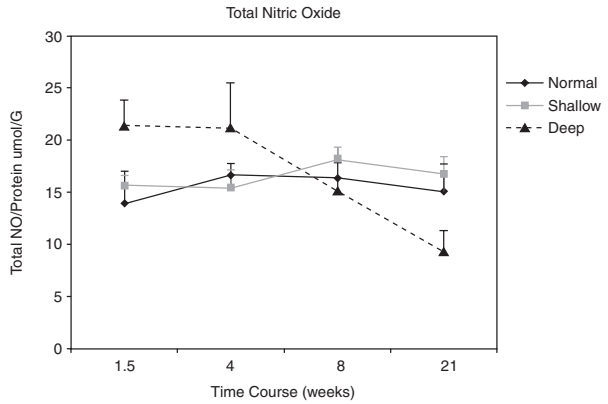
CHANGES IN NITRIC OXIDE LEVELS AFTER DEEP DERMAL INJURY IN THE FEMALE, RED DUROC PIG (FRDP)

KQ Zhu MD, LH Engrav MD, PM Muangman, MD, JK Cole, MD, NS Gibran MD
Department of Surgery, University of Washington, Seattle, WA.

Introduction: Hypertrophic scar is a devastating sequel to burns and other tangential skin injuries. It follows deep dermal injuries and does not occur after superficial injuries. Nitric oxide (NO) plays many important roles in wound healing from inflammation to scar remodeling. Studies have shown that expression of nitric oxide synthase and nitric oxide production are decreased in human hypertrophic scar. However little is known about NO involvement in the early stages of hypertrophic scarring, because of the lack of an animal model. It was recently reported that the female red Duroc pig (FRDP) makes thick scar, which is similar to human hypertrophic scar. We hypothesized that NO production in wounds on the female, red Duroc pig is similar to that of human hypertrophic scar and that NO involvement in deep wounds is different from that in superficial wounds.
Methods: Superficial (0.015" to 0.030") and deep (0.045" to 0.060") wounds were created on the backs of four FRDPs. Biopsies were collected at weeks 1.5, 4, 8 and 21 post wounding including samples of uninjured skin. Nitric oxide levels were measured with the Griess reaction assay and normalized with tissue protein level.
Results: Superficial wounds healed with an invisible scar whereas the deep wounds healed with scar resembling mild hypertrophic scar. The thickness of the scars from the deep wounds was significantly greater than uninjured skin and healed superficial wounds ($p < 0.01$). NO levels were increased at 1.5 weeks in deep wounds compared to superficial wounds and uninjured skin ($p < 0.05$). At 8 weeks, NO levels in deep wounds had returned to the level of uninjured tissue and superficial wounds. By 21 weeks, NO levels had decreased significantly when compared to superficial wounds ($p < 0.01$). There were no differences in NO levels between uninjured skin and superficial wounds at any time point ($p > 0.05$).
Conclusions: NO production is similar in late, deep wounds on the female, red Duroc pig to that reported in the literature for human hypertrophic scar further validating this animal model. NO production is quite different after deep wounds as compared to superficial wounds in the FRDP. Early elevation in nitric oxide production might account for excessive inflammation in deep wounds that become thick scars in the FRDP. Nitric oxide regulators and effects at early stages of scar formation should be elucidated further and the FRDP appears to be a useful model.



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LENTIVIRUS AND ADENO-ASSOCIATED VIRUS MEDIATED TARGETED GENE TRANSFECTION IN LOW-OXYGEN ENVIRONMENT

Nakshatra K. Roy (n-roy@northwestern.edu), Sheng-Xian Jia, Thomas A. Mustoe
Wound Healing Research Laboratory, Department of Surgery, Northwestern University, Chicago, IL 60611, USA.

A critical limitation of using retroviral vectors for gene therapy is their inability to infect non-dividing cells. Although, the adenoviral vectors have the advantage of being able to infect both dividing and non-dividing cells, they elicit inflammatory response, thus making the interpretation of *in vivo* experiments harder. Adeno-associated virus (AAV) and Lentiviral vectors do not have those limitations, however, scant information is available about their transfection efficiency under low-oxygen tension. To determine if low-oxygen microenvironment affects viral vector-mediated gene transfection, we have used two other viral vectors, Adeno-associated virus (AAV) and Lentiviral constructs in vitro and in vivo to express foreign genes in hypoxic cultured human dermal fibroblasts and ischemic rat wounds. Both cultured normoxic and hypoxic (1% O_2) human dermal fibroblasts were identically transfected by the AAV vector. A lenti6-LacZ construct was injected onto the periphery of rat ischemic and non-ischemic wound (10^6 pfu/wound) at the time of wounding. Wounds were harvested at post-operative day 7. Frozen sections of the wounds were fixed in cold acetone and stained with a in situ β-gal staining kit. Intense expression of β-gal was observed without any inflammatory response. No significant difference of transfection efficiency was observed between the ischemic and non-ischemic wounds. Thus our data indicates that both AAV and Lentiviral vectors are suitable to use in gene-therapy experiments in both ischemic and non-ischemic cells and tissues in vitro and in vivo.

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EXAMINING REGULATION DIFFERENCES IN TGFB1 WITH AGE AND ISCHEMIA USING THE RAT BACK FLAP MODEL

P. S. A. Procaccini, S. Jia, K. Cross, N. Roy, J.E. Mogford, T.A. Mustoe
Wound Healing Research Laboratory, Division of Plastic Surgery, Northwestern University, Chicago, IL 60611

Chronic wounds are characterized by a continued inability to heal after an extended period of time (often defined as 3 weeks or longer) and more commonly found in the elderly over areas of compromised blood flow. Collagen is a gene vital to this healing process and Transforming Growth Factor beta 1 (TGFβ1) is an important signaling molecule for collagen induction. We chose to examine the effects of age and ischemia on TGFβ1 regulation using the rat backflap model, which demonstrates negative influences of age and ischemia on the wound healing. Four full-thickness biopsy punches (7 mm) were made centered on the back of aged and young rats. The rostral wound pair was made ischemic by raising a transverse flap (1.8 cm wide × 8 cm length). The caudal wound pair served as a non-ischemic control. Wound contraction was prevented by insertion of a sterilized polyethylene sheet under both the ischemic and nonischemic wounds. RNA was then extracted from wounds at post operation days 3, 7, 10 and 14. The RNA was then reverse transcribed and assayed by Real-Time PCR to examine the relative expression of TGFβ1. TGFβ1 expression in the ischemic wounds of young rats was significantly increased versus nonischemic controls at post-wounding days 3, 7 and 14 ($p < 0.05$, $p < 0.05$, $p < 0.05$, respectively). We also observed a significant increase in TGFβ1 expression in the ischemic of aged animals at day 14 ($p = 0.01$). The data suggest that there is either a loss of function in TGFβ1's response to ischemia in the aged animals, or, at least, a significant delay. The results also demonstrate the usefulness of the rat backflap model for the study of age and ischemia on TGFβ1 function in a non-contractile rat wound model.

This work was supported by NIH Grant 5R01GM041303–13 (TAM).

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HIF-I AND VEGF REGULATION IN ISCHEMIA

P. S. A. Procaccini, S. Jia, N. Roy, J.E. Mogford, T.A. Mustoe
Wound Healing Research Laboratory, Division of Plastic Surgery, Northwestern University, Chicago, IL 60611

Epithelialization and vascularization are key elements involved in the repair of cutaneous wounds. One method of regulation for these pathways is expression of a signaling molecule known as Vascular Endothelial Growth Factor (VEGF), which, in turn, is regulated by the transcription factor Hypoxia-Inducible Factor-1 (HIF-1). It has been suggested that chronic wounds, which are most commonly located in ischemic tissue among the elderly, may be the result of a loss of function in the VEGF regulation pathway. Using our rat back flap model, which demonstrates the negative influences of age and ischemia on wound healing, we examined the relative expression of HIF-1 and VEGF in aged and young rats at 3,7, and 10 days post-wounding. Four full-thickness biopsy punches (7 mm) were made on the back of each aged and young rat. The rostral wound pair was made ischemic by raising a transverse flap (1.8 cm wide × 8 cm length). The caudal wound pair served as a non-ischemic control. Inserting a sterilized polyethylene sheet under both ischemic and nonischemic wounds prevented wound contraction. RNA was extracted from the tissue at the specified time points, and run on Real-Time PCR to determine relative expression of VEGF and HIF-1. Preliminary data suggest that, at the 3 day time point, there is a significant increase in both HIF-1 and VEGF expression in young rats under ischemic conditions and a significant increase in VEGF expression in aged rats under ischemia with a strong (but not significant) trend towards increase in HIF-1 as well. While the rat model does not produce significant differences between aged and young animals, this upregulation under ischemia indicates that HIF-1 and VEGF expression may play a vital role in the early signaling of ischemic wound repair.

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USE OF EPR SPECTROSCOPY TO MEASURE TISSUE OXYGEN IN AN ISCHEMIC FLAP MODEL

LJ Gould[1], J Mushkudiani[1], H Hou[2], OY Grinberg[2], S Grinberg[2], HM Swartz[2]
[1]University of Texas Medical Branch, Galveston, TX [2]EPR Center for Viable Biological Systems, Dartmouth Medical School, Hanover, NH

Methods to reliably measure tissue oxygenation in situ are currently lacking. We have developed a vertically oriented, dorsal, bipedicle flap model that is easy to perform, reliably reproduces tissue ischemia, eliminates craniocaudal variation, and is amenable to studying therapeutic modalities. The effect of narrowing this flap on tissue oxygenation measured with Licox electrodes has previously been presented. In this study we utilize in situ EPR spectroscopy to demonstrate the oxygen gradient in the flap as a function of flap width and placement of a silicone sheet directly under the flap. The effect of wound healing over a 2 week period is demonstrated.
Twenty four, 300 gm male Sprague-Dawley rats underwent creation of the bipedicle flap according to the following groups: 2.5 cm flap with silicone, 2.0 cm flap without silicone, 2.0 cm flap with silicone. Each group of 6 animals was injected with EMS char at 2 cm intervals along the flap and one injection in the control, non-ischemic tissue. A 4th group underwent 2.0 cm flaps with silicone and use of lithium phthalocyanin as the paramagnetic material. Wound measurements and EPR spectroscopy were performed on days 3, 7, 10 and 14. On day 14, after EPR measurements, the animals were sacrificed and their wounds excised. One flap and one control wound were preserved for histologic analysis, the other flap and control wounds were prepared for lactate measurements.
EPR spectroscopy demonstrated a gradient of oxygen that was lowest in the center of the flap and greatest at either end. Changes in the oxygen gradient correlated with narrowing and placement of the intervening silicone sheet. This new technology has never been utilized in an animal model of impaired wound healing. Comparison of recently developed paramagnetic materials for optimal tissue oxygen and free radical measurements will be presented.

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A NEW ANTIMICROBIAL ALGINATE DRESSING FOR MODERATE TO HEAVILY EXUDING, INFECTED AND CRITICALLY COLONISED WOUNDS

Deborah Addison, Tracy Jane Rennison, Sally-Anne Norris,
Johnson & Johnson Wound Managment

Aim: An antimicrobial dressing that combines high absorbency and potent antimicrobial properties. The unique composition of the dressing manages exudates in moderate to heavily exuding, infected or critically colonised wounds, which creates a favourable environment for effective wound management.
Methods: Two test methods were used to evaluate the antimicrobial properties of the dressing: 1) A zone of inhibition sensitivity test that challenges the dressing to a seeded agar plate and 2) A log reduction test that exposes a small sample of dressing against a high bacterial loaded broth, measuring the log reduction of the bacteria over a specified time period. The absorbency of the dressing was evaluated using British Pharmacopoeia test methods.
Results: The antimicrobial dressing has been shown to be highly effective against a wide range of microorganisms, including Pseudomonas aeruginosa, Escherichia coli, Streptococcus pyogenes, Staphylococcus aureus, Staphylococcus epidermidis, Klebsiella pneumoniae and Candida albicans and also against resistant organisms MRSE, MRSA and VRE. The product is highly absorbent with absorbency > 16 g/g and 16 g/m^2.

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HB-50: A PRE-CLINICAL STUDY OF A PROPHYLACTIC FOR WOUND INFECTION

Scott M Harris[1], Lijuan Zhang[1], Jody Parente[1], George T. Rodeheaver[2], and Timothy J. Falla[1].
[1]Helix BioMedix Inc., Bothell Washington 98021
[2]University of Virginia Plastic Surgery Research, Charlottesville, VA 22908

Topical prophylaxis against wound infection by an agent that is active against multi-resistant bacteria does not generate resistance and is rapidly cidal would be of great clinical benefit. Peptides of the innate immune system have long been known to protect a wide range of organisms from attack by bacterial and fungal pathogens. Helix BioMedix Inc. has developed a short bioactive peptide antimicrobial modeled after these peptides. HB-50 is an amphipathic cationic alpha-helical peptide that has broad spectrum activity and is rapidly microbicidal. These attributes make HB-50 an ideal candidate for wound infection prophylaxis. In vitro studies have demonstrated HB-50 to be active against both gram-positive and gram-negative bacteria killing 5–7 log orders of bacteria within minutes. In addition, this peptide has potent activity against Vancomycin and Mupirocin resistant *S. aureus*. Preliminary testing of the peptide in a rat abraded skin infection model has shown the peptide's effectiveness in preventing wound infection while not inhibiting wound healing. Additionally, the HB-50 sequence has been specifically developed to be cost effective to manufacture and therefore is well suited for use as a topical antimicrobial agent.

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EFFECTS OF HIGH VOLTAGE PULSED CURRENT ON BACTERIAL VIABILITY: AN IN VITRO STUDY

Cassie A. Kuykendall, SPT, Trish L. Hutchinson, Ed.D., FACSM, Alan P. Bloedaum, Ph.D.
Angelo State University, San Angelo, Texas

Background and Purpose: High voltage pulsed current (HVPC) has been shown to have positive effects on wound healing due in part to proposed bacteriocidal effects in wound beds. The purpose of this study was to examine the effects of electrical stimulation (HVPC) using in vitro *Streptococcus A* and clinically-accepted wound care guidelines.
Methods: Commercially available *Streptococcus A* was grown overnight in Trypticase Soy Broth. Standard 100 millimeter (mm) Petri dishes of Trypticase Soy Agar with 5% sheep blood were then streaked using sterile cotton swabs. 52 plates were subjected to 45-minutes of HVPC with the following parameters: 120 pps, 200 V, 10-second on/off cycle, and 2-second ramp time. Negative polarity was used as the active electrode. 48 control plates were prepared identically without the application of the HVPC. Zones of inhibition were measured the following day at the positive and negative electrode sites to the nearest millimeter after a 24-hour incubation period using the Kirby-Bauer method.
Results: Differences were calculated using a two-way ANOVA with SPSS 11.5. Mean inhibition at the positive and negative electrodes was $0.00\,\text{mm} \pm 0.00$ and $10.13 \pm 4.13\,\text{mm}$, respectively. Significant between group effects were found for the control and experimental groups ($F_{1,199} = 289.4$, $p < 0.001$), negative and positive electrodes ($F_{1,199} = 289.4$, $p < 0.001$), and interaction of groups by electrodes ($F_{1,199} = 289$, $p < 0.001$).
Conclusion: In vitro, negative polarity HVPC using clinically accepted and feasible parameters can inhibit *Streptococcus A* growth around the negative (active) electrode. No growth inhibition was noted around the positive (dispersive) electrode. This could provide an alternate means of infection control in assistance with wound healing.

Acknowledgments to Mr. Mark Pape and Dr. Carol B. Diminnie

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DOES WOUND CARE EDUCATION SPEED HEALING OF INFECTED, ACUTE WOUNDS?

J Sherman, MD, A Lyndon, RNC, MS, CNS, H Hopf, MD,
University of California at San Francisco, Wound Healing Laboratory

Background: Wound infections cause significant morbidity in women after C-Sections. Regular lectures on wound care were instituted for obstetrical residents at our institution in 1999. We hypothesized that resident education would decrease time to complete closure in post-C-Section wounds healing by secondary intention.
Methods: With IRB approval, a retrospective chart review was performed to obtain the following information in patients treated pre (1996–1999) versus post (2000–2003) education intervention: age, race, occupation, social support, medical co-morbidities, medications, week of gestation at delivery, duration of 1st stage of labor, length of ruptured membranes, meconium, chorioamnionitis, Group B Strep status, indications for C-Section, antibiotics, wound treatment, wound culture, wound dressing, time to full closure, number of visits for wound care (home, office), and need for repeat procedures.
Results: Data are currently being analyzed.
Conclusions: If resident education reduces the time to full closure, then we will evaluate expanding this teaching approach to other departments using a randomized prospective study.

Sponsored by NIH NIGMS 27345.

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A COMPARISON OF *IN VITRO* DISC DIFFUSION AND TIME-KILL KINETIC ASSAYS FOR THE EVALUATION OF ANTIMICROBIAL ACTIVITY OF SILVER CONTAINING WOUND DRESSINGS

Corrie L. Gallant[1], Hua Q. Yin[2], Rita E. Langford[2], Merle E. Olson[1], David A. Hart[1] and Robert E. Burrell[3]
1. Department of Microbiology and Infectious Diseases, University of Calgary, Alberta, Canada
2. Nucryst Pharmaceuticals Corporation, Fort Saskatchewan, Alberta, Canada
3. Department of Chemical and Materials Engineering, University of Alberta, Edmonton, Alberta, Canada

There is a plethora of new silver containing dressings on the market today. Various manufacturers attempt to show that their dressings are the most efficacious and therefore should be preferentially employed by health care workers based on the results of their *in vitro* tests. However, there have been no studies to date that clearly identify which *in vitro* tests are appropriate for comparison purposes. The available literature suggests that there are problems with disc sensitivity assays in terms of assessing the antimicrobial efficacy of silver. Spadaro (1985) has shown that the diameters of zones of inhibition were not proportional to the concentration of anodic silver in complex test media. Further, Richards et al (2001) have shown that zones of inhibition did not appear to correlate to log reduction assay data for silver containing dressings. Other authors favour log reduction or time kill assays as they better compare to clinical data. The purpose of this study was to determine which *in vivo* test is most appropriate for evaluating the antimicrobial efficacy of silver-containing dressings. This was done by testing 8 different silver containing dressings and 2 non-silver (mafenide acetate or hexamethylene biguanide) containing topical agents against 17 clinically relevant microorganisms using both zone of inhibition assays and time-kill kinetic assays in complex media. The results for the two assays were then correlated to determine if the methods generated similar results. It was determined that the two methods do not correlate at all. This is most likely a result of the silver interacting with the media in the zone of inhibition test, thus invalidating the results of this test. We therefore conclude that zone of inhibition data generated for silver-containing dressings is of little value when assessing antimicrobial efficacy, and that time-kill assays are of greater use.

Funding provided by Natural Sciences and Engineering Research Council of Canada (NSERC) and Nucryst Pharmaceuticals.

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CARBOHYDRATE-BASED WOUND DRESSINGS WITH ELASTASE-LOWERING ACTIVITY

Vincent Edwards[1], Dorne Yager[2], Kelman Cohen[2], Steven Goheen[3],
(1) Southern Regional Research Center, New Orleans, LA, (2) MCV-VCU, Richmond VA, (3) PNNL, Richland WA,,

The use of carbohydrate-based wound dressings including cotton, xerogels, charcoal cloth, alginates, and hydrogels, for chronic wounds have afforded properties such as absorbency, ease of application and removal, bacterial protection, fluid balance, occlusion, and elasticity. An additional consideration is the use of the dressing to regulate proteolysis in the non-healing wound. Elastase is a serine protease that has been associated with a variety of inflammatory diseases, and recently has been implicated as a destructive protease that impedes wound healing. The presence of elevated levels of elastase in non-healing wounds has been associated with the degradation of important growth factors and fibronectin necessary for wound healing. Here we consider the design, preparation, and assessment of carbohydrate-based wound dressings that either release protease inhibitors or act as sequestering agents to bind proteases such as elastase and thus reduce their high levels in the chronic wound. Carbohydrates ranging from small monosaccahrides up to large polysaccharides are crosslinked onto cotton wound dressings. The resulting carbohydrate conjugates of cotton cellulose possess unique properties advantageous for chronic wound treatment ranging from gelation and elasticity to elastase-sequestering properties
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RISK FACTORS FOR PRESSURE ULCERS IN ELDERLY HOSPITAL PATIENTS

Mona Baumgarten, D. Margolis, R. Lowe, R. Localio, S. Kagan, J. Holmes, W. Kavesh, B. Kinosian, S. Abbuhl, A. Ruffin

Elderly hospital patients have ample exposure to situations and procedures that may increase their pressure ulcer risk. The aims of this study were to estimate the incidence of hospital-acquired pressure ulcers in elderly medical patients and to determine whether the risk of pressure ulcers is associated with longer emergency department (ED) stays, lengthy procedures in the ED, or use of immobilizing restraints, medications, or devices in the ED. The study cohort was made up of 3,233 patients aged 65 or over admitted through the EDs of two Philadelphia hospitals. Sixty-five percent of subjects were aged 80 or more, 61% were female, and 69% were Black. The incidence of hospital-acquired pressure ulcers on Day 3 of the hospital stay, as ascertained by direct skin examination, was 6.2%. There were 266 hospital-acquired pressure ulcers among 201 patients (mean per patient 1.3 pressure ulcers) 27% of the pressure ulcers were stage 1, 54% were stage 2, and the remainder were stages 3 or 4 or could not be staged. The median length of stay in the ED was 6.6 hours. The association between hospital-acquired pressure ulcers and extrinsic risk factors will be presented. The identification of potentially modifiable risk factors may lead to the development of effective interventions to prevent hospital-acquired pressure ulcers.

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CARE OF THE THERMALLY INJURED BREAST

R.E. Ortega, MD, L.H. Riina, MD, T.A. Davenport, MD, L.T. Glickman, MD, R.L. Simpson, MD, K.M. Burger, PA, D. Hangan, RN

Nassau University Medical Center, 2201 Hempstead Tpk, East Meadow, NY 11554, rorte ga007@hotmail.com

Introduction: Thermal injury to the female breast is one of the most challenging aspects of aesthetic burn surgery today. As the ability to provide coverage for large body surface injuries has progressed greatly in recent years; attention can now be directed towards achieving aesthetically pleasing results. Breast reconstruction in the burn patient can be accomplished in several ways. In planning the reconstruction, one must account for gender, age, and stage of breast development at the time of injury. The following is the treatment protocol utilized at our institution. After determining the level of injury the injured area is cleansed and treated with topical anti-microbial agents such as Silvadene. If the decision is made to excise and graft the injured area one of two algorithms is followed. The first choice involves excising the burn and placing a split thickness skin graft to the area involved. This is done by placing a sheet graft and using aerosolized fibrin sealant to affix it to the wound bed. If the burn involves deeper elements of tissue then a second approach is taken which includes excision of the burn down to the level of fascia with preservation of the breast mounds and the nipple areola complex (NAR). The (NAR) is spared excision and allowed to heal. Reconstruction of the (NAR) can be deferred for a secondary procedure depending upon the response to primary healing. A split thickness skin graft is then applied to the area of injury. Again a sheet graft is preferred and fibrin sealant is utilized to improve graft fixation and contour. We attribute our excellent results to the sheet grafts and fibrin sealant used. It should be noted that the increased vascularity of the breast fat when compared to fat located elsewhere in the body allows the grafts to adhere and survive on this generally difficult to graft surface.
Methods: We identified five female patients at our institution over the last 18 months with thermal injuries to the breasts. Each patient was placed into one of the two treatment algorithms.
Results: The five patients had excellent outcomes. Breast mounds and symmetry were preserved. Further development of the breast was allowed in each patient. One patient even underwent a breast augmentation after surviving a 50% TBSA injury. Proper use of fibrin sealant and sheet grafts account for the excellent results seen at this institution.
Conclusion: Following careful evaluation of the burned female breast cosmetically and functionally acceptable results can be attained when following our institution's protocol for breast reconstruction in the female burn victim.

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DERMAGRAFT AS AN ADJUNCT IN THE TREATMENT OF VENOUS LEG ULCERS

R.J. Snyder

Medical Director, Wound Healing Center at University, Tamarac, Fl USA

Aim: To document successful healing of a recalcitrant venous ulcer with adjunctive use of Dermagraft
Materials and Methods: A 74-year-old Hispanic male presented with a chronic venous ulcer at the medial aspect of his right ankle. Biopsy failed to reveal malignancy and confirmed stasis changes. Treatment was ongoing including compression therapy, local debridements, and moist wound healing, off-loading, and nutritional support. The wound remained free of infection. Due to the continued recalcitrant nature of the lesion multiple applications of Dermagraft were utilized to augment healing.
Results: The researcher observed dramatic improvement in wound dimensions, wound contracture, and granulation tissue.
Conclusions: Dermagraft appears to augment healing in patients with venous ulcers and may be useful as adjunctive therapy.
Acknowledgements: Smith & Nephew Wound Management, Largo, Fl.

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CLINICAL EXPERIENCE WITH THE SELF-ASSEMBLED SKIN SUBSTITUE AS A BIOLOGICAL DRESSING FOR CHRONIC VENOUS LEG ULCERS TREATMENT

N. Dubé[1], M.-H. Rochon[1], C.E. Ospina[1], J. Soucy[2], L. Germain[1], F.A. Auger[1]

[1]LOEX, CHAUQ, Hôpital du Saint-Sacrement, Université Laval, Québec, Canada [2]CHAUQ, Hôpital de l'Enfant-Jésus, Québec, Canada

Introduction: Venous ulcers are the most common chronic wounds of the lower limbs. In our laboratory, the self-assembly method is used to reconstruct autologous skin that can be used as a biological dressing (*In Vitro Cell Dev Bio Anim*, 1999: 35, 318; *J Chem Eng*, 2001: 79, 663).
Goals and Methods: The aim of this study was to establish if our self-assembled skin substitute (SASS) allows the closure of nonhealing venous leg ulcers. A case study of a 68-year-old woman with a chronic venous leg ulcer present for over one year and measuring 135 cm^2 is thus herein presented. The autologous cells were isolated from a cutaneous biopsy (1 cm^2) and cultivated *in vitro* (*J Chem Eng*, 2001: 79, 663). The autologous SASS was produced and assembled by the cells thus it does not contain any exogenous or synthetic material (*In Vitro Cell Dev Bio Anim*, 1999: 35, 318). The wound was debrided, covered with grafts of SASS and maintained in place with a compressive dressing (Coban, 3M, St-Paul, MN). A new SASS was applied weekly until wound closure. The SASS seemed to lessen the pain caused by the ulcer. Seven applications of the SASS were necessary to lead to a full wound closure.
Conclusion: Venous leg ulcer quickly reepithelialized after application of our SASS This case indicates that use of the SASS can reduce pain and promote closure of nonhealing leg ulcers Furthermore, we thus hope that our SASS biological dressing maybe clinically useful to treat difficult-to-heal leg ulcers. This study was approved by the Health Canada's Therapeutic Products Directorate and by the Ethic Committee of the CHAUQ, Saint-Sacrement Hospital.
Acknowledgements: Fédération des Pompiers du Québec pour les Grands Brûlés.

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

Kekan M. and Feldman D.,

Department of Biomedical Engineering University of Alabama at Birmingham, Birmingham, AL, USA

The standard clinical assessment parameter for skin wounds has been time to heal. There is a need for assessments that monitor the healing process and have been validated as indicators of clinical outcome. 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. Since this measure can have a high variability, previous studies were done to determine the key environmental and operational parameters as well to determine the correlation between blood perfusion and tissue oxygen levels. This helped determine the parameters to study as well the utility of using the change in blood perfusion after a provocation (e.g. heat) rather than the absolute blood perfusion level.
The objectives of this study were to further determine operational parameters, to develop clinical protocols, and to begin the process of correlating blood perfusion values to clinical outcome. The effect of parameters such as light intensity, distance of the laser head from the skin, and skin pigmentation on blood perfusion were examined. Further, the relationship between skin temperature and blood perfusion was studied as well as the best ways to deliver this provocation clinically. Preliminary studies have helped determine the appropriate ranges for each parameter to give the most consistent readings. In addition, the best way to use the heat provocation has also been determined. In ongoing studies similar experiments are being carried out on pressure ulcers to assure these protocols work for clinical wounds. The best clinical protocols will then be used to validate the prognostic capabilities of the LDPI in terms of predicting wound healing rates (overall healing rate, epithelialization rate, and contraction rate).
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Acknowledgements: Biofisica, LLC and CDC through NCIPC

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EFFICIENT SEQUESTRATION OF NEUTROPHIL-DERIVED PROTEASES BY A NOVEL POLYMERIC HYDROGEL

D.J. Vachon[1], D.R. Yager[2]
[1]Aegis BioSciences, LLC, Granada Hills, CA USA [2]Virginia Commonwealth University, Richmond, VA USA

There is a growing interest in the development of wound dressings that possess functionality beyond providing physical protection and optimal moisture environment. To this end, a novel dressing material based on a sulfonated-hydrocarbon backbone has been developed for the controlled release of therapeutic agents into chronic wounds. This versatile polymer possesses an ion-exchange capability that makes it amenable for binding and releasing a variety of potential agents. Polymer backbone was coated onto polyester fabric and then modified by ion exchange to incorporate Na^+, Ag^+, or doxycycline onto the polymer. The ability of several formulations of this hydrocarbon backbone product to sequester elastase and neutrophil collagenase (MMP-8) was then examined.
Wound fluids containing elastase and MMP-8 were obtained from pooled wound fluids obtained from consented patients with pressure ulcers. Equivalent weights of product formulations were incubated for 2–4 hours at 25 °C with the wound fluids and then the remaining activities of elastase and MMP-8 determined using specific substrates (methoxysuucinyl-ala-ala-pro-val-p-nitroanilide and collagen type I respectively). Untreated polyester fabric served as a control and weight equivalents of gauze and Promogran® were also included for the purposes of comparison. The average reduction in apparent elastase activity in the wound fluid was 4%, 20.4%, 14%, 29%, and 55% for gauze, Promogran®, doxycycline, Ag^+, and Na^+ formulations respectively. Only Promogran® and the doxycycline formulation affected a decrease in apparent collagenolytic activity.
These preliminary results provide evidence in support of the versatility of these new biomaterials for the controlled modulation of the chronic wound environment.

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MICROVASCULAR DRESSING FOR BURN APPLICATIONS

M. Cabodi[1], A.D. Stroock[1], L. Staiano-Coico[2], Roger Yurt[2]
[1]Chemical Engineering, Cornell University, Ithaca, NY USA [2]Weill Medical College, Cornell University, New York, NY USA

Vascular structures are a ubiquitous element of living tissues that allow for efficient mass transfer with the bulk of the tissue. Reasons for the vascularization of tissue include: 1) efficiency of transport over large distances (>1 mm) by convection, 2) spatial control of delivery and evacuation of solute from the tissues, and 3) centralization of the influx and efflux from the tissue; this centralization allows for temporal control and monitoring to be performed. Many of these characteristics would also be valuable in synthetic materials designed for interaction with biological systems. We will present the design and fabrication of a wound dressing that incorporates microvasculature in the form of embedded microchannels. As in living systems, the basic principle behind our design is that active convection is used to carry solute over large distances, while diffusion acts over smaller distances.
We will describe the microfabrication procedure, based on soft-lithographic techniques, used to fabricate the microvascular dressing. We will also discuss the design parameters which are important for the optimization of mass transfer and a model of the system will be presented. We will report on the physical characterization of the system, with respect to the mass transport properties. Finally, we will discuss initial considerations about the use of these structures as wound dressings.

This material is based upon work supported by the STC Program of the National Science Foundation under Agreement No. ECS-9876771.

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A NEW STUDY DESIGN TO COMPARE WOUND DRESSINGS

Baldur Baldursson MD PhD
Dermatologist University Hospital Reykjavik Iceland

The initial tests of a new ulcer dressing, even if the materials are well known and safe must include comparison with other dressings.
In order to test the performance of a new dressing manufactured to treat medium to heavily exuding wounds we designed a wound model using wounds resulting from harvesting of punch grafts for treatment of chronic ulcers. These are usually made by a 6 mm wide biopsy punch and a scalpel and are 7 mm wide round split skin wounds reaching down to the reticular dermis in a number from 2–300.
10 consecutive patients with leg ulcers that had a punch graft operation performed at the in-patient Dermatology ward in Reykjavik, Iceland were enrolled. The grafts were harvested on the antero-lateral aspect of the thigh in a pattern of three groups each with 3–20 punch graft wounds. A different type of wound dressing was used to treat each group. Dressing changes were scheduled for one, four, 12 and 18 days after the operation.
The nurse graded the appearance of the split skin wounds into infected or non-infected, inflammatory or non-inflammatory. The patient graded the degree of pain at dressing removal using a visual assessment scale.
The dressings were weighed before and after to assess fluid absorption. Detailed digital photographs recorded the progress of each group of donor sites.
The outcome of the different parameters seemed consistent and repeatable.
Using the donor site of punch grafts is a repeatable and consistent way of studying the performance of new ulcer dressings, comparing them to established dressings.

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VENOUS ULCER HEALING PREDICTED BY URINE NITRATE ASSAY: A RETROSPECTIVE STUDY

J.V. Boykin[1], C. Baylis[2], D. Yager[3], I.K. Cohen[3], V. Sommer[1], L. Shawler[1], M. Watkins[1], J. Young[1], M. Crossland[1],
[1]Retreat Hospital Wound Healing Center, [3]Div. Plastic Surgery, Virginia Commonwealth Univ., Richmond, VA, [2]Dept. of Physiology, West Virginia Univ., Morgantown, WVA

Chronic venous stasis ulcerations (VSU) are associated with significant platelet and leukocyte activation with secondary superoxide anion and ROS production that may severely degrade nitric oxide (NO) production. This study was designed to document the possible association between NO production and the impaired healing of VSU patients. Twenty-four subjects were used for the study. Group C ($n = 8$) the control group, consisted of healthy adults without history of VSU. Group HU ($n = 8$) the healed ulcer group, consisted of adults with documented VSU whose ulcers healed with routine wound care and compression therapy in 20 weeks or less. Group UU ($n = 8$) the unhealed ulcer group, consisted of adults with documented VSU without healing within 20 weeks. All groups had normal renal function and were hospitalized for 24 hours on bed rest with a low arginine and low nitrate diet. Fasting samples were obtained for plasma and urine nitrate (NOx) and plasma arginine, asymmetric dimethylarginine (ADMA) and isoprostane determinations. At 24-hrs, group HU demonstrated the highest plasma NOx levels ($33.25 \pm 11.69\,\mu M$) as compared to group C ($19.45 \pm 2.63\,\mu M$) or group UU ($27.17 \pm 3.67\,\mu M$); urine NOx excretion of group UU ($51.46 \pm 11.77\,\mu M$) was significantly lower ($p < 0.05$; Kruskal-Wallis) as compared to group HU ($135 \pm 38.29\,\mu M$) or group C ($164.3 \pm 38.58\,\mu M$). ADMA and arginine were not significantly different between groups. Ratios of NOx to isoprostane were disproportionate between groups. These results suggest, for the first time, a significant relationship between impaired VSU healing and deficient NO production. Furthermore, these findings establish the clinical value of measurements of NO production and bioavailability as effective predictive-biological markers for the healing of VSU.

Supported by a grant from Pfizer Limited

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"MIST ULTRASOUND THERAPY FOR RECALCITRANT WOUNDS, A NEW APPROACH"

William J. Ennis[1], Wesley Valdes[2], Patricio Meneses[3].
[1]Advocate Christ Medical Center, Wound Treatment Program, Medical Director [2]Wound Care Fellow, Advocate Christ Medical Center, Wound Treatment Program [3]Research Director, Advocate Christ Medical Center, Wound Treatment Program

The use of adjunctive peri-wound, ultrasound with either a 1 MgHz or 3 MgHz frequency has been reported on in the literature with variable results. MIST ultrasound therapy is a novel application of direct wound bed application of kilohertz ultrasound waves utilizing a normal saline mist as the coupling mechanism, allowing for no direct patient contact. The mist generated by the system is of relative uniform particle size and acts as a conduit of ultrasound energy. Unlike ultrasonic baths, which dissipate the vibrational energy over a large area, the transducer horn focuses the energy into a smaller area of application.
The MIST™ system consists of an ultrasonic power supply (generator), a transducer (or alternately referred to as the converter), and an applicator coupled to the transducer. The generator converts voltage to high frequency electrical energy. This electrical energy is transmitted to the piezoelectric transducer within the converter, where it is changed to mechanical vibrations. The generator is designed to operate the converter at 40 kHz with a distal displacement of 60 microns. Published reports have described various biological effects of low frequency ultrasound including VEGF and PDGF production, increased protein synthesis, improved bone and wound healing. Other authors have published abstracts demonstrating clinical efficacy of this device on a broad range of patients with recalcitrant wounds. This report describes the clinical results of a prospective, non-randomized, efficacy trial, adding MIST ultrasound therapy to current treatment protocols in recalcitrant wounds. Wound healing trajectories before and after treatment will be evaluated and wound healing will be assessed with laser Doppler image analysis and trans-cutaneous oximetry.

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SCROTAL AND PENILE RECONSTRUCTION USING VAC THERAPY

N Snyder[1], LJ Gould[1]
[1]University of Texas Medical Branch, Galveston, TX

Fournier gangrene is a rapidly progressive necrotizing soft tissue infection that often leaves patients with a large integumentary deficit to their penis and scrotum. Treatment of the skin defect has been described in a multitude of ways including split thickness skin grafting, burying the testes in the thighs, thigh flaps, and a variety of myocutaneous and fasciocutaneous flaps.
Many of the previously described techniques have worked well for smaller defects and for closure of wounds but fell short of aesthetic reconstruction of sexual organs. We describe a technique using split-thickness skin grafts with suction dressings or bolster. This was employed using the VAC© (Vacuum Assisted Closure Device, KCI©). This technique creates a natural appearing scrotum that holds the testes away from the body in a physiologic manner and surfaces the penis with a seemingly natural appearance in a functional manner. The technique provides a nearly 100% graft take and greatly decreases hospital length of stay. A case report demonstrates a patient's full recovery to his professional, social, and sexual life within six weeks of initial reconstructive treatment. Our technique provides a reliable treatment with good functional and aesthetic outcomes to a difficult problem.

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THE IMPORTANCE OF ARM DOMINANCE IN ACUTE WOUND HEALING

T. Serena, on behalf of the Wound Healing Cooperative Group
Wound Healing Cooperative Group, The Angiogenesis Foundation, Cambridge, MA USA

Background: Physiological differences between dominant and non-dominant arms may play an important role in wound healing of the upper extremities. The Wound Healing Cooperative Group (WHCG) studied this phenomenon in a human forearm model of acute wound healing.
Methods: With informed consent, sixteen normal healthy volunteers underwent four 6 mm biopsies on the flexor surface of both forearms: 2 biopsies on the dominant arm, and 2 on the non-dominant arm. The acute wound sites were then treated with i) rhPDGF-BB 0.01% gel topically at various dosing intervals (Q.D. versus Q.O.D.), ii) rhPDGF-BB alone Q.D. $\times$ 7 days followed by bacitracin alone, or iii) bacitracin alone. Time-to-complete healing was measured as the primary endpoint.
Results: The average time-to-complete healing for the dominant arm was shorter than the time required to heal the biopsies on the non-dominant arm, irregardless of the treatment applied.
Conclusion: In the forearm biopsy model of acute wound healing, arm dominance is an independent variable and must considered in study designs. We postulate that this effect may be due to variation in microvascular perfusion. These results provoke further investigation into the physiological mechanisms underlying increased rate of healing in the dominant extremity.
Acknowledgements: Funding from The Angiogenesis Foundation.

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WOUND HEALING MORPHOLOGY IN AN ACUTE WOUND HEALING STUDY

T. Serena, on behalf of the Wound Healing Cooperative Group
Wound Healing Cooperative Group, The Angiogenesis Foundation, Cambridge, MA USA

Background: Physiological healing in acute wounds generally occurs by granulation from the wound base and by migration of epithelium from the wound edges toward the center of the wound in a uniform, concentric fashion. The Wound Healing Cooperative Group (WHCG) compared this phenomenon in acute wounds treated with standard care versus acute wounds pharmacologically stimulated with growth factor therapy.
Methods: With informed consent, 20 normal healthy volunteers underwent four 6 mm biopsies of the flexor surface of both forearms. The biopsy sites were randomly assigned to a control arm (daily bacitracin) or to one of three treatment arms: i) rhPDGF-BB 0.01% gel Q.D., ii) rhPDGF-BB Q.O.D., iii) daily rhPDGF-BB (Q.D. $\times$ 7 days followed by bacitracin alone daily). Wound morphology was carefully examined and photographed daily until complete healing was achieved.
Results: There were distinct differences in the morphological pattern of healing seen between control wounds and growth factor-stimulated wounds. Acute wounds treated with bacitracin tended to heal in circumferential fashion, as predicted. Growth factor-stimulated wounds, by contrast, exhibited accentuated angiogenesis (granulation), with non-uniform epithelial islands streaming into the wound.
Conclusion: This pilot study suggests that rhPDGF-BB influences acute wound healing by promoting accelerated granulation and epithelialization. Accelerated healing is manifest by different healing morphologies. The biological basis for these differences requires further histological and molecular analyses.
Acknowledgements: : Funding from The Angiogenesis Foundation.

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A SUMMARY OF SCIENTIFIC STUDIES AND CLINICAL RESULTS WITH A GLYCERINE BASED DRESSING

E.I. Stout
Southwest Technologies, Inc., N. Kansas City, MO, USA

The importance of controlling the bioburden in wounds can not be over emphasized. Glycerine based gel sheets have been used extensively to show their bacteriostatic/fungistatic properties. Hoekstra studied animal wounds and compared glycerine dressings with water based dressings and the glycerine showed superior bioburden reduction. Vandeputte showed similar results when comparing hydrogel and hydrocolloid dressings and looked at the histology of the wounds to find differences in the quantities of the types of cells present. The reduced scar formation of wounds are thought to be attributed to the influence of the glycerine on the healing process. Hoestra has reported the dramatic reduction in the inflammation reaction soon after application of the glycerine gel dressing. Studies by Oliveria-Gandia, Davis, and Mertz showed the glycerine dressings to be more effective than hydrogel or hydrocolloid dressings in reducing bioburden in animal wounds that were inoculated with microbes and also reducing biocounts in appropriate growth medium. Vandeputte conducted a diabetic study(no exclusions) that compared the glycerine dressing ($n = 15$) with standard protocol($n = 14$) for diabetic foot wounds, that showed the test dressing to be far superior. He along with thousands of other nurses around the world have reported the use of glycerine dressings on superficial burns to reduce pain, reduce the chance for infection, reduce scar formation, and to protect the wound from friction and pressure. J. Baksa extensively used the glycerine gel sheets in his burn unit not only for the superficial wounds but also for 3^{rd} and 4^{th} degree burns on children as well as after surgical removal of hypertrophic and keloid scars to prevent reoccurrence. T.M. Baum and M.J. Busuito also reported the use of the glycerine dressing for scar prevention and treatment. The glycerine dressing has been used extensively for te treatment and prevention of pressure ulcers in hospitals, nursing homes, athletic fields, as well as, under casts, splints and braces. R. Horchner reported a >95% reduction in pressure ulcers in a direct comparison to the control and to hydrocolloids.

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DEVELOPMENT AND CHARACTERIZATION OF A SKIN ORGAN CULTURE THAT RESEMBLES HUMAN SKIN

M. Martins-Green, QiJing Li, Min Yao.
Department of Cell Biology and Neuroscience. University of California Riverside, Riverside CA, 92521.

The inability to experiment in humans creates a need to develop culture systems that mimic human tissues/organs. Skin is arguably the simplest tissue hence provides an excellent prototype for tissue engineering. Moreover, skin is the body's first line of defense. Currently available skin replacements have a number of drawbacks. Therefore, there is a need for skin replacements that: (a) are prepared with pertinent primary human cells but yet can be ready "off" the shelf; (b) can be prepared rapidly; (c) contain stable structures, in particular microvessels that can rapidly connect with the patient's vasculature, thereby establishing circulation in the "graft" and increasing the chances of survival; (d) can be tailored for specific wound impairments (e) are long lasting. We have developed a generation human "skin" that can fulfill these requirements and can potentially be used as a "living bandage". We start with three primary human cell types and a collagen matrix that self-assemble into a connective tissue containing a network of mature microvessels, is covered with a stratified epidermis, expresses biochemical markers, matrix molecules, and cytokines characteristic of normal human skin, and matures in 10–15days. Moreover, two additional cell types, pericytes and monocytes, differentiate *in situ* adjacent to and within microvessels, respectively. The epidermis expresses keratins typical of mature skin and not characteristically produced in response to injury such as keratins 6, 16, 17. This tissue potentially can be developed into a skin replacement for patients with impaired healing. In addition, it responds normally to biological stimuli, providing a powerful vehicle to investigate mechanisms of skin development and regeneration, understand pathological processes, and test drugs and treatments for skin diseases.

Supported by AHA and TRDRP.

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LATERAL WICKING, GOOD OR BAD

Baldur Baldursson
University Hospital Reykjavik Iceland, Christopher Collins, Ossur hf Reykjavik.

The ability of polyurethane sponge dressings to absorb and contain wound fluid is often insufficient, resulting in maceration and eczema.
The term *lateral wicking* has been used to describe the fluid film between the peri-ulcer skin and the dressing surface, responsible for the maceration. A "within dressing" lateral wicking is where the wound fluid is absorbed above the ulcer and transported laterally within the dressing without affecting the peri-ulcer skin.
We wanted to test this phenomenon in a new type of dressing with a silicon surface towards the wound, a polyurethane sponge layer with enclosed absorbent particles and a vapour permeable outer layer.
A pump delivered 10 ml per hr of equine serum to a 1 sq cm surface at the centre of the dressings. The experiment went on for 2.5 hours. Seven dressings of each type were used in the experiment. The top and bottom aspects of the dressings were photographed at 30 min interval.
In the new ulcer dressing the serum was absorbed and distributed in a "within dressing" lateral wicking. In the established dressing an "under dressing" lateral wicking took place leading to erratic distribution of absorption often at the margin of the dressing instead of over the centre.
Conclusion
Enhancing lateral wicking within a chronic ulcer dressing is a realistic way of optimising its fluid handling characteristics. In this experimental set-up the new ulcer dressing performed as intended while the conventional dressing did not.
Improved fluid handling characteristics are needed in today's advanced dressings however, the importance of this phenomenon in the clinical situation must be tested in clinical trials.

Ossur hf producing company of Gentleheal founded the study

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DIFFERENTIAL REGULATION OF OSTEOPONTIN AND MATRIX METALLOPROTEINASES DURING DIABETIC WOUND HEALING

A. Sharma, A. K. Singh, J. Warren, R. K Maheshwari
Uniformed Services University of the Health Sciences, Bethesda, Maryland, USA

Delayed wound healing is a common complication of diabetes resulting in significant clinical morbidity. The diabetic wound exhibits impaired cellular infiltration and consequently inadequate granulation tissue formation. Additionally, altered patterns of apoptosis. In this study, we have evaluated the differential gene expression pattern in transgenic diabetic female mice, 5–6 weeks old, in a full thickness cutaneous punch wound model. We assessed the role of matrix metalloproteinases (MMPs) and differential gene expression pattern at 4th, 7th and 11th day post wounding. Supernatants obtained from diabetic wound tissue homogenates were subjected to zymogram analysis. The data showed that MMPs were expressed at higher level by 4th day post wounding, whereas expression of MMPs were down regulated towards the 11th day post wounding suggesting their role during early phase of wound healing.
The pathway specific gene array data demonstrated differential regulation of several growth factors, transcription factors and other related genes such as fibroblast growth factors and their receptors, ID3 and restin respectively. The cytokine/extracellular matrix protein osteopontin (OPN), an important component of cellular immunity and inflammation also showed higher expression after 4 days post wounding. The expression of OPN remained at higher level after 11 days post wounding in diabetic mice, whereas the expression were down regulated to basal level in normal wounded animal suggesting that the expression of OPN was concomitant with the extent of healing. Other adhesion molecules such as integrin αV and PECAM-1 were also differentially regulated. Though a single gene may not be solely responsible for any defect or impairment in healing as it is a very tightly controlled and regulated process, however, a detailed study of these gene(s) may shed some light to the delayed healing in diabetic mice.
Acknowledgements: (These studies are supported by NIH Grant G174KT.)

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GRANULATION TISSUE INDUCTION BY LASSAR OINTMENT VS. COLLAGEN-POLYVYNILPYRROLIDONE IN VENOUS ULCERS

Edgar Krötzsch[1], Arturo Suárez-Colín[2], Rosa M. Salgado[1], Ana M.Z. Apis[3],.
[1]Connective Tissue Laboratory, División de Investigación Biomédica, C.M.N. "20 de Noviembre", I.S.S.S.T.E. Mexico City, Mexico. [2]Plastic and Reconstructive Department, Hospital General de Zona 32, and [3]Research Area, Delegación 3 y 4, I.M.S.S. Mexico City, Mexico.

Venous leg ulcers derived from tissue destruction is the consequence of a chronic inflammatory process that produces pain and physical disability, diminishing quality of life in patients. In this work, Lassar ointment and lyophilized collagen-polyvinylpyrrolidone were administered separated each on one half in the same ulcer to 9 patients at the beginning and every 4 days. On day 16, all patients were auto-grafted with partial thickness skin. Granulation tissue and graft integration were assessed clinically during 3 months. Inflammatory infiltrate, type I and III collagens, elastic fibers, alkaline phosphatase as well as blood vessels were evaluated histologically or histochemically in biopsies taken at the beginning and 16 days after the local treatment.
Clinically and morphologically, both treatments demonstrated appropriate granulation tissue promotion and optimal graft integration since the beginning. Nevertheless, in Lassar ointment treated group regionalization of alkaline phosphatase activity was observed, as well as the presence of granuloma in 2 of the 9 patients. In conclusion, Lassar ointment or lyophilized collagen-polyvinylpyrrolidone are two different promoters of granulation tissue in venous leg ulcers, however Lassar ointment has the capability to produce granuloma and an exacerbated immune response; in consequence, ulcer recidivism could be present, may be due to mineral deposits in the wound.

This work was partially supported by Aspid S.A. de C.V.

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EVALUATION OF ALTERATIONS IN GENE EXPRESSION IN NORMAL AND DIABETIC MICE RESULTING FROM DERMAL INJURY

Kathleen E. Rodgers, Ph.D., Dolph D. Ellefson, Ph.D., Theresa Espinoza, M.D., Ya-hsuan Hsu, M.S., Gere S. diZerega, M.D. Ruty Mehrian-Shai, Ph.D.
University of Southern California KECK School of Medicine

C57Bl/6-db/db mice are a model of delayed healing after full thickness dermal excision injury. We evaluated the differences in gene expression between C57Bl/6 mice and C57Bl/6-db/db mice in uninjured skin and within a wound 7 days after injury and observed a number of differences resulting from the diabetic mutation. Cluster analysis indicated differences in genes involved in all structural organization and metabolism. There were 718 genes that had greater than 2 fold difference between these 2 strains of mice in granulation tissue at day 7. In normal and diabetic mice, 884 or 765 genes were altered 2 fold by injury (granulation tissue), respectively. When compared at the 4 fold level, the overlap of genes altered between normal and diabetic mice was approximately 68%. In granulation tissue, changes were observed in genes associated with inflammation, immune response, cell motility, and response to external stimuli. Normal mice had greater immune, stress, inflammatory, and biotic responses. Wounds of diabetic mice had increased responses in energy metabolism and fatty acid metabolism. These data provide a basis for further understanding of the response of skin to injury and the delay that occurs in insulin resistant Type 2 diabetes.

American Diabetes Association.

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INDUCTION OF GENE EXPRESSION FOR EGF-LIKE LIGANDS IN PORCINE WOUNDS FOLLOWING RECEPTOR OVEREXPESSION

M.I. Okwueze, N.L. Cardwell, A.C. Pollins, L.B. Nanney
Vanderbilt University, Nashville, TN USA

A rationale to explain the role of the large and redundant family of EGF cytokines with multiple receptor combinations has proven elusive. We examined endogenous gene expression levels during wound repair for four EGF-like cytokines (Transforming growth factor-alpha (TGFα), Heparin-binding epidermal growth factor (HB-EGF), Epiregulin (EPR) and Betacellulin (BC) using a quantitative real-time PCR technique. In addition, cytokine gene expression was evaluated after overexpression of different of EGF receptor forms (erbB-1, erbB-3 and erbB-4) or a control B-galactosidase gene using an adenoviral transfection strategy to introduce either homo or heterodimer receptor combinations. At baseline, endogenous gene expression levels of TGFα and HB-EGF were 10-fold and 100-fold higher than EPR and BC, respectively. Expression of TGFα was increased by 3-fold in the receptor combination of erbB-1/erbB-4 expressing wounds. Recent studies on the EGF-related polypeptides HB-EGF, EPR and BC have shown preferential binding to erbB-4. Our study suggests that wounds predominantly expressing erbB-4 showed a 2.5-fold increase in gene expression of HB-EGF. A synergistic 4-fold increase in gene expression was noted when erbB-4 overexpression was treated with HB-EGF ligand. In contrast, wounds transfected with erbB-4 alone showed no change in EPR gene expression compared to controls. However, wounds treated with TGFα showed 3-fold increase in EPR gene expression and EPR potentiated itself. Similarly, wounds transfected with erbB-4 and treated with growth factors TGFα and EGF upregulated the gene expression of BC by 4-fold as compared to controls. Interestingly, wounds overexpressing erbB-3 with topica application of EGF accentuated expression of TGFα by 7-fold. In histologic sections, these wounds showed a 2-fold increase in the dermal region compared to control. Thus, the EGF-like family of ligands and its homo- or heterodimer receptor pairs have the potential in vivo to evoke differing profiles or intensities of gene expression patterns as they mediate wound repair.

(This project is funded by the NIH GM40437)

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CELOSIA ARGENTEA LINN. LEAF EXTRACT IMPROVES WOUND HEALING IN RAT BURN WOUND MODEL

Kulasekaran S Priya[1, 2], Mary Babu[2] and Alan Wells[1].
[1]Departments of Pathology, University of Pittsburgh, Pittsburgh, PA.USA [2]Biomaterials Division, Central Leather Research Institute, Chennai, TamilNadu, India.

Celosia argentea is used in traditional medicine for sores, ulcers, and skin eruptions. Therefore, we investigated the healing efficiency of an ointment formulated on a alcohol extract of *Celosia argentea* leaves (CA) in a rat burn wound model. Wound closure occurred earlier in the treated rats (15 days versus 30 in the untreated group; $P < 0.05$). Collagen and hexsoamine content of the granulation tissue increased at a faster rate in the treated wounds. To probe the cell biologic basis of this effect, we found that this extract promoted cell motility and proliferation of primary dermal fibroblasts but did not alter these responses in primary keratinocytes. In short, we demonstrate a salutary action of the *Celosia argentea* extract on wound healing and suggest that this may be due to mitogenic and motogenic promotion of dermal fibroblasts.

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THE CUTANEOUS "UNDERLYING DISORDER" IN DIABETIC RATS

S.L. Lu C. Qing T. Xie M.J. Wang Y.W. Niu K. Ge S.W. Jin Z.J. Liao J.X. Shi

Shanghai Burns Institute, Rui Jin Hospital, Shanghai second Medical University, 200025 Shanghai, China

Objective To investigate the histological characteristics and pathophysiological changes in diabetic skin.
Methods: 12 Sprague-Dawley rats weighing 200–220 g were divided into control and STZ-induced diabetic groups. The shaved skin specimens from the back of rats were collected on 8 w post STZ-inducing. The cutaneous histological characteristics were observed. The local contents of glucose, advanced glycation end products(AGEs) and hydroxyproline, the levels of aMMP-2 and TIMP-2, and the cell cycles of both keratinocytes and dermal cells were determined.
Results: The thicknesses of epidermis layer and dermis layer were both reduced obviously in diabetic skin, with the morphological characteristics of the obscured multilayer epithelium features and the decreased amount of spinous in epidermis; the atrophied, swollen and degenerated collagen fibers with a focal chronic inflammatory cells infiltration. The results also revealed that contents of glucose, AGEs, the level of aMMP-2 and the ratio of aMMP-2/TIMP-2 in diabetic skin were higher than those in the controls. In diabetic group, the percentages of S stage and G2/M stages of keratinocytes were obviously decreased, while the dermal cells showed the higher percentage of S stage and the normal percentage of G2/M stages, when compared with the control group.
Conclusion: The cutaneous histological and pathophysiological alterations in diabetes mellitus has already been occurred when exogenous damage is not existed, which presented an "underlying disorder" characteristics in diabetic skin. The cutaneous "underlying disorder" in diabetes might be a critical risk factor leading to ulceration and one of the most important mechanisms in the pathogenesis of impaired wound healing.
[Key words]: diabetic skin underlying disorder cell proliferation MMPs collagen

The project is supported by: Major State Basic Research Development Program of China (No. G1999054205).

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MOLECULAR PATHOGENESIS OF CHRONIC WOUNDS: THE ROLE OF β-CATENIN AND C-MYC

O. Stojadinovic[1], H. Brem[2], B. Lee[1], C. Vouthounis[1], H. Entero[2] and M. Tomic-Canic[1].

[1]Department of Dermatology, NYU School of Medicine, New York; [2]Department of Surgery, Mt. Sinai School of Medicine, New York

The lack of understanding of molecular pathogenesis of impaired healing in chronic ulcers leads to a serious health issue that contributes to excessive limb amputations and mortality. Using biopsies from patients with chronic wounds, skin organ culture and primary keratinocytes in culture we identified that β-catenin and its downstream target, c-myc, play important role in development of chronic wounds. In contrast to normal epidermis, we observed significant nuclearization of β-catenin and elevated c-myc expression at the non-healing wound edge of patients with chronic ulcers. In vitro studies indicated that activation and stabilization of nuclear β-catenin inhibits wound healing and keratinocyte migration by: blocking EGF response and inducing c-myc. Using Affymetrix large scale microarrays we found that β-catenin downstream target, c-myc, is induced in skin by an inhibitor of wound healing (glucocorticoids) and repressed in the initial phase of normal wound healing, 4 to 48 hr, whereas it becomes de-repressed at 96 hr post wounding. Therefore, the activation of β-catenin/c-myc pathway(s) contributes to impaired healing by inhibiting of keratinocyte migration and altering keratinocyte differentiation. The presence of activated β-catenin and c-myc in the epidermis of chronic wounds may serve as molecular markers of impaired healing and future targets for therapeutic intervention. While β-catenin signaling has been implicated in epithelial development and oncogenesis its role in wound healing has never been postulated. This further illustrates the importance of "tissue context" specificity, because β-catenin in the context of malignant tissue promotes invasion whereas in the context of a wound environment does the opposite.

Our research is supported by NIH grants AR45974, NR08029, DK59424, DK43093; EPVA and ADA.

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GENE ARRAY PROFILING OF KELOID FIBROBLASTS TO IDENTIFY THE TARGET GENES FOR THERAPEUTIC EVALUATIONS

Latha Satish, Jian-Hua Luo, Kulasekaran S Priya and Alan Wells

Department of Pathology, University of Pittsburgh, PA 15261

Keloids are scars that overgrow the original boundaries of the injury, involving aberrant functioning of the fibroblasts. We have found that during normal wound healing the ELR-negative CXC chemokines IP-9 (CXCL11) and IP-10 (CXCL10) act to limit fibroblast immigration. Our initial goal was to determine if keloid fibroblasts failed to respond to this 'stop' signal. Immunohistochemical and immunocytochemical studies on keloid fibroblasts show that these lesions express excessive amounts of IP-9 and CXCR3 receptor. The protein levels of CXCR3 receptor was also not dissimilar between normal and keloid fibroblasts. Though, the expression of the protein and the receptor seems to be normal, the fibroblasts derived from these abnormal lesions did not respond to IP-9 during EGF induced cell migration. We have extended these findings to determine whether other genes are differentially expressed by keloid fibroblasts. The immediate first gene that interest us was the mRNA levels of CXCR3 receptor (GPR9) wherein, we did not find any significant difference between normal and keloid fibroblasts. Further, we also found interestingly, that genes related to tumor progression are up regulated in keloid fibroblasts than the normal fibroblasts. The marker genes like calgranulin, BCL-2 associated anthogen 4 (BAG4), and dual specificity phosphatase 1 (DUSP1). Certain transcription factors like GATA-6 and SpiB are also increased. Apart, the classical growth factor and growth factor receptor related genes which includes, bone morphogenetic protein BMP-1, heparin binding EGF like growth factor, EGF like repeats discoidin1, IGFBP-5 and 3, EphB1&B2, tumor necrosis factor alpha induced protein, protein tyrosine phosphatase receptor, serine threonine kinase 6 along with chondroitin sulfate proteoglycan brevican and versican and vitronectin are also up regulated. The newl identified genes will open new avenues in targeting keloid lesions by understanding the basic signaling mechanism of these specific proteins.

This work was supported by grants from NIH/NIGMS and PPRTP.

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MICROARRAY EXPRESSION ANALYSIS OF FETAL MOUSE SKIN DEVELOPMENT: IMPLICATIONS FOR SCARLESS HEALING

R Yun, W Kong, R Faudoa, W Xia, TM Krummel, MT Longaker, and HP Lorenz,

Pediatric Surgical Research Program, Department of Surgery, Stanford University

Introduction: Fetal mouse skin wounds heal without scar before gestational day E17. Because scarless repair is inherent to fetal skin and occurs superimposed on skin differentiation, genome-wide gene expression during skin development was tested.
Methods: Dorsal skin from BALB/C mice fetuses at E14, E15, E18 and E20 was collected. RNAs from individual fetuses were hybridized to mouse microarrays with 42,000 gene elements. The E14/E18 hybridizations were repeated three times, and the E15/E20 repeated twice with different samples. ("x" = fold change).
Results: Increased genes on E18 and E20 were clustered into several groups: 1) ECMs: type I (2.8x), III (2.3x), VI, and XIV procollagens; 2) Cell surface: integrin beta1 binding protein2 (5.2x), integral membrane protein2A and 2B (avg 3.8x); 3) Proteases: mast cell protease4 (15x) and 5 (24x), MMP23 (3x); 4) Growth factor-related: acidic FGF (3.7x), FGF receptor3 (2.9x), TGF-alpha (2.3x).
Decreased genes on E18 and E20 included: 1) Growth factors: TGF-beta2 (0.42x), PDGF-alpha (0.41x), PDGF receptor-beta (0.37x), NGF receptor associated protein (0.18x); 2) Proteases: MMP 11 (0.27x), 14 (0.39x); 3) ECM: fibromodulin (0.5x), Vcam1 (0.22x), keratin18 (0.2x) and 19 (0.46x), collagen XVIII (0.36x).
Conclusions: Genes with increased expression at E14, E15 and decreased expression at E18, E20 have possible antifibrotic function. These data identify hundreds of possible antifibrotic and pro-fibrotic genes as candidates for further functional analysis as regulators of repair.

Supported by NIH DE00463–03

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HB107: A PRE-CLINICAL THERAPEUTIC PEPTIDE

Scott M Harris[1], Lijuan Zhang[1], Jody Parente[1], Richard L. Gallo[2], Phillip H. A. Lee[2], and Timothy J. Falla[1].

[1]Helix BioMedix Inc., Bothell Washington 98021
[2]University of California, San Diego, 92122

Peptides of the innate immune system play a vital role in the protection and repair of almost all biological systems. Such peptides have been implicated in a range of activities associated with prevention of disease and modulation of innate immunity. HB107 is a derivative of one such peptide, Cecropin B, that has demonstrated efficacy in enhancing wound healing in both burn and incision animal models. HB107 has been evaluated for efficacy in a mouse incision model and for safety and efficacy in a pig burn wound model. Topical application of the peptide gives RE^{50} (time needed for 50% re-epithelialization) values of 10.28 days for 500 ug/mL and 12.72 days for 100 ug/mL compared to control 16.45 days. Additionally the peptide was well tolerated in terms of safety both topically and in an IV acute toxicity mouse model with no adverse effects observed. HB107 not only demonstrated efficacy and safety, but due to being a relatively short synthetic peptide, costs significantly less to manufacture than the current approved therapies.

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β-CATENIN AND CARM-1 AS CO-REPRESSORS OF GLUCOCORTICOID RECEPTOR LEAD TO INHIBITION OF KERATINOCYTE MIGRATION

O. Stojadinovic[1], C. Vouthounis[1], B. Lee[1], M. Stallcup[2] and M. Tomic-Canic[1].

[1]Department of Dermatology, NYU School of Medicine, New York; [2]Department of Pathology, University of Southern California, Los Angeles

Ability of keratinocytes to migrate relays on appropriate citoskeletal network and is one of the key prerequisites of wound healing. Keratins K6 and K16 are the cytoskeletal components that mark migrating keratinocytes. We found K6/K16 to be differentially regulated in microarray experiments: induced in the wound healing and suppressed by inhibitors of wound healing, glucocorticoids (GC). We have shown previously that GC suppress K6/K16 expression through a unique molecular mechanism that involves four monomers of glucocorticoid receptor (GR). Because β-catenin interacts with members of the nuclear receptor family, such as retinoic acid receptors (RAR) and androgen receptor (AR) and participates in both activation and repression of transcription and similarly to GC inhibits keratinocytes migration we hypothesized that β-catenin participates in GC-mediated repression of K6/K16. To test if β-catenin participates in K6/K16 suppression by GC we used co-transfection experiments, with primary human keratinocytes. By itself, β-catenin did not affect K6/K16 expression. However, in the presence of GC, β-catenin acted as a GR co-repressor further suppressing K6/K16 expression. Moreover, protein arginine methyltransferase CARM-1 enhances this co-repression, suggesting that the complex that suppresses K6/K16 promoters contains GR, β-catenin and CARM-1. Similarly, β-catenin functioned as a co-repressor of GR even if we just incubated keratinocytes with LiCl (stabilized β-catenin) rather than transfected its expression plasmid. It has been shown that β-catenin and CARM-1 bind each other in a co-activator complex with AR, but it was not known if this complex has a co-repressing capacity. We have shown that by participating in the GC-mdiated repression of K6/K16 transcription as a co-repressor with CARM1, β-catenin contributes to the inhibition of keratinocyte migration through altering the cytoskeletal network and subsequent inhibition of wound healing.

Our research is supported by: AR45974, NR08029 and EPVA.

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HB-107, A NON-ANTIMICROBIAL FRAGMENT OF CECROPIN B, ACCELERATES MURINE WOUND REPAIR

P. H. A. Lee[1], S. M. Harris[2], L. Zhang[2], T. J. Falla[2], and R. L. Gallo[1]

[1]Dept of Medicine, Div of Dermatology, UCSD, and VA Hospital, San Diego, CA USA; [2]Helix BioMedix Inc., Bothell, WA USA

Intact skin represents the first line of defense against microbial pathogens. Effectors of innate immunity such as antimicrobial peptides are important when this barrier is breached due to infection or injury. Antimicrobial peptides can kill microbes directly, however several investigations have suggested they can also modify host cell behaviors to promote wound repair. It is unclear whether the ability of these molecules to positively influence wound repair is dependent on their antimicrobial function or on their ability to influence the host. To investigate this, the microbial killing capacity of specific antimicrobial peptide fragments was determined and compared with their ability to affect wound repair in a murine model of aseptic full-thickness excisional injury. HB-107, a peptide fragment derived from Cecropin B, lacks antimicrobial activity yet showed up to 64% improvement in wound closure at day13 when compared to either scrambled peptide or vehicle controls. This effect was comparable to that seen after treatment with currently accepted therapy. Histological evaluation of wounds treated with HB-107 displayed keratinocyte hyperplasia and increased leukocyte infiltration compared to controls. To explore the mechanism for these findings, we tested the ability of HB-107 to stimulate IL-8 secretion. HB-107 peptide was able to stimulate IL-8 release from cultured dermal microvascular endothelial cells when compared to scrambled peptide control or other peptides derived from Cecropin B. Taken together, this data confirms that antimicrobial peptides can function as important effectors of wound repair independent of antimicrobial function.

Study was funded by a Helix Biomedix, Inc. grant, a VA Merit Award (RG), NIH grants AR-45676 and AI-052453 (RG), and the Generalist Physician-Scientist Training Program NIH-NCI 1T32 CA81211 (PL).

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THE UPREGULATION OF WOUND HEALING RELATED GENES IN VENOUS ULCERATIONS TREATED WITH APLIGRAF, A POSSIBLE MECHANISM OF ACTION

T.E. Serena, C. Finley

Gannon University; Penn North Centers for Advanced Wound Care, 552 Quaker Hill Rd. Warren, PA 16365 USA

Background: Apligraf, a bioengineered living skin construct, has been shown to accelerate the healing of chronic venous leg and diabetic foot ulcerations. However, to date the mechanism of action of Apligraf in the wound healing process is not well understood.
Objective: The primary objective of this study was to determine the levels of expression of selected wound healing related genes in venous leg ulcers treated with Apligraf in comparison to ulcers treated with standard multi-layer compression therapy alone. Gene chip technology was employed.
Methods: Three patients were randomized into the Apligraf or standard treatment arms. A baseline 6 mm punch biopsy was obtained prior to the initial application of Apligraf or multi-layer compression therapy. A second biopsy was obtained depending on the randomly chosen biopsy schedule (weeks 1, 2 or 4 following Apligraf application or initiation of compression). The biopsy specimens were snap frozen and later analyzed using microarray gene chip technology.
Results: The patients treated with Apligraf demonstrated an up-regulation of the genes thought to be important in the wound healing process.
Conclusion: The results from this pilot study suggest that Apligraf may function by an up-regulation of the genes involved in wound healing as opposed to compression therapy which works primarily in a mechanical fashion. Based on these results further study is needed.
Acknowledgements: : Funding from Organogensis Inc.

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BIOLOGICAL EFFECTS OF CALRETICULIN ON WOUND REPAIR

L.I. Gold, J. Michaels, M.J. Callaghan, R.D. Galiano, M. Rahman, G.C. Gurtner, L.B. Nanney
New York University School of Medicine, New York, NY USAVanderbilt University School of Medicine, Nashville, TN USA

Calreticulin (CRT) is a major classic Ca-binding chaperone protein of the endoplasmic reticulum. Recently, CRT has been recognized to have widespread extracellular effects as well. In the current study we show that CRT increases both epithelial migration and granulation tissue formation in models of porcine and murine wound repair. Partial thickness wounds were created on the paravertebral area of pigs ($n = 4$) and 0.1% and 0.5% CRT, and PDGF (positive control) applied for 4 consecutive days. In wounds harvested at 5 days, CRT induced a 28 and 22% greater extent of reepithelialization than PDGF and Tris/Ca buffer control, respectively (% healed = 56/CRT; 40.5/PDGF; 44/control). In addition, CRT stimulated earlier granulation tissue formation in a dose-dependent manner (cumulative dermal depth, microns: 1615/CRT; 1250/PDGF; 1325/control). A similar granulation tissue inducing effect of CRT was also observed in a steroid-impaired pig model. As a diabetic model of wound repair, two 5 mm circular full-thickness wounds were created on the dorsum of db/db mice; the wounds were splinted open with silicone rings and covered with occlusive dressing ($n = 24$). After 5 days of treatment with 0.1, 0.5, and 5% CRT, a dose-dependent 8-, 4.5-, and 2-fold increase in granulation tissue formation was observed ($p < 0.05$). However, there was no apparent effect on wound closure. Tissue sections showed a highly cellular dermis in the CRT treated wounds. In addition, CRT (50 pg/ml) stimulated wound closure in a scratch plate assay using fibroblasts by 45%, in 48 hrs, compared to 2% for the control. Therefore, CRT may be a novel agent for wound healing by acting as a chemoattractant for cells involved in wound remodeling and in epithelial migration.

Suppported by Calretex, LLC. NJ, USA (LIG) and the Alumni Fund, Alumni Association Downstate College of Medicine (MJC).

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ENHANCING THE REPAIR QUALITY OF INJURED SKIN ON PORCINE AFTER AUTOGRAFTING WITH BONE MARROW MESENCHYMAL STEM CELLS

Xiaobing Fu Lijun Fang, Yuxin Wang, Tongzhu Sun, Biao Cheng.
Wound Healing and Cell Biology Laboratory, Burn institute, 304th Hospital, Trauma Center of Postgraduate Medical College, Beijing 100037, P.R.China (fuxb@cgw.net.cn)

To explore the effects of bone marrow mesenchymal stem cells (MSCs) on the quality healing of porcine skin wounds after burn injury so as to provide a new method for clinical skin repair in the future. Seventy-two deep-partial thickness burn wounds were produced on the back of 6 minipigs and randomly divided into 6 groups: saline control, MSCs treatment, MSCs plus bFGF treatment, MSCs plus EGF treatment, bFGF treatment or EGF treatment only. MSCs were isolated from porcine marrow and cultured in vitro. After labeling with BrdU, MSCs were autografted onto the skin wounds. At 7, 14, 21 and 42 days after injury, the area of the wounds were measured and the histological examination was performed to evaluate the velocity and quality of wound healing. At 1, 2 and 4 weeks after transplantation, immunohistochemical examinations were carried out to detect the positive staining of BrdU, cytokeratin and S-100 to evaluate the wound healing quality. The area of wounds was decreased at day 7 and most of these wounds were healed on day 21 after injury. There was no significant difference on the contraction rate among six groups. Histological examination demonstrated that the number of vessel and the expression density of S-100 in MSCs plus bFGF treatment wounds were significantly enhanced than that in other groups. MSCs autografting may benefit to enhance the wound healing quality in porcine skin, which may open a new way to reach a "perfect repair" after skin injury.

This study was supported in part by the National Natural Science Foundation of China (30170966, 30230370), National Basic Science and Development Programme (973 Programme, G1999054204)

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PERSISTENCE AND EXPANSION OF SEEDED MARROW CELLS IN DERMAL SUBSTRATE

PY Liu, XT Wang, C Ponte, Tang, JB, EV Badiavas
Roger Williams Medical Center, Providence, RI USA
Boston University Medical Center

Bone marrow cells have been used to potentiate wound healing in chronic wounds (Badiavas and Falanga, 2003). We postulate that the performance of these autografted cells may be enhanced by seeding them into scaffolding with normal skin architecture; indeed that the differentiation of the precursor cells may be determined by the juxtaposition of intact extracellular matrix. This might in turn lead to a prefabricated skin substitute capable of recapitulating fully functional skin.
We are seeding murine gfp+ bone marrow cells, containing mixed mesenchymal and hematopoietic precursor cells into Alloderm®, a decellularized preserved skin graft. This seeding is potentiated by meshing the skin and gentle centrifugation of fresh marrow into the dermal aspect of the allograft. Other samples were merely co-cultured with the cells in Dexter's media. Duplicate samples were fixed and stained with DAPI at 1, 3, 7, and 14 days. Viewed under fluorescence, gfp+ cells are bright green.Comparing these images with DAPI-staining allows viable cells to be identified which are gfp+. H&E micrographs were used to ascertain cellular morphology.
Results: Scant cells were observed in the allografts at the early time points. The cells were pleiomorphic with large nuclei, resembling hematopoietic precursors(Fig 1). Later, at days 7 and 14, more abundant cells were apparent, including some in the interior of the dermis. This signifies either migration or seeding via centrifugation. The morphology of these cells is significantly different; the spindle shape suggests a fibroblast (Fig 2). In addition, the population of gfp+ cells has markedly expanded by day 14, attaining near confluence in some sections (Fig 3). No cells were seen in controls (Fig 4).
Conclusion: True skin replacement will only be possible when the panoply of skin appendages can be recapitulated. The experiments presented here are evidence that pluripotential precursor cells can be seeded into dermal matrices providing an optimal environment for regeneration.

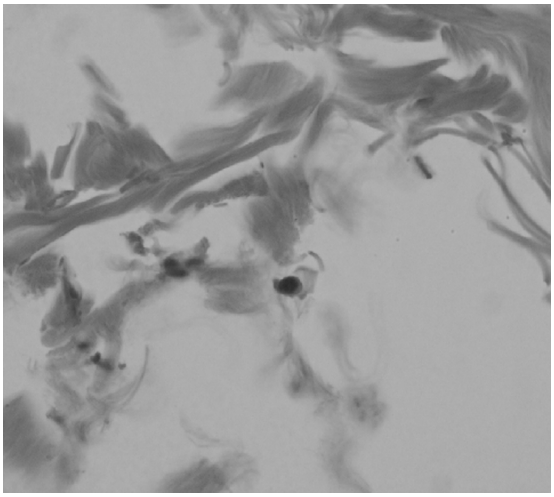

Fig. 1.

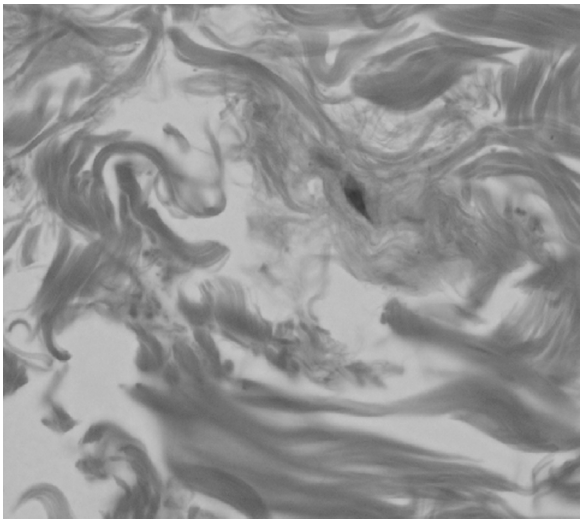

Fig. 2.

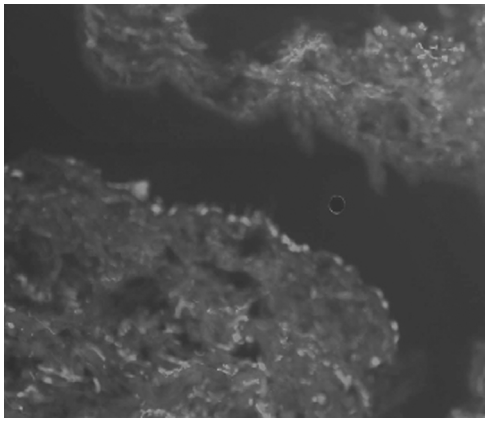

Fig. 3.

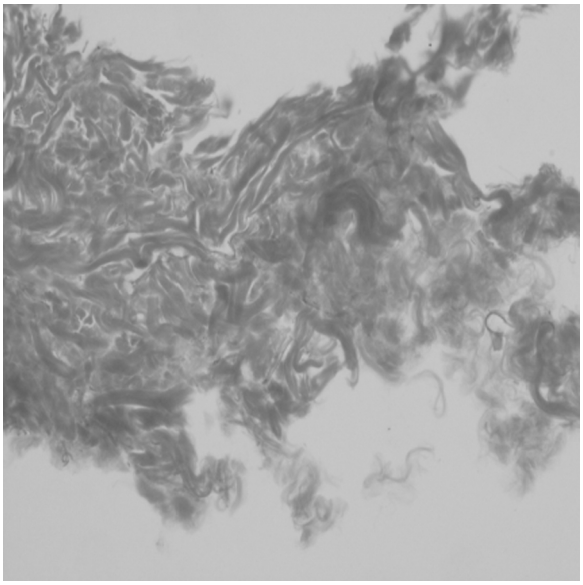

Fig. 4.

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2-METHOXYESTRADIOL, AN ENDOGENOUS ESTROGEN METABOLITE, REGULATES APOPTOSIS IN SKIN MICROVASCULAR ENDOTHELIAL CELLS

A. Philip, A-M Horvat and M. Chowdhury
Department of Surgery, McGill University, Montreal, Quebec, Canada

Programmed cell death or apoptosis regulates the sequence of cellular events that are involved in the wound healing process. Understanding the mechanisms controlling apoptosis during tissue repair may lead to the development of modalities to improve healing and reduce scarring. 2-Methoxyestradiol (2ME), a naturally occurring metabolite of 17- estradiol, has recently emerged as a promising anti-proliferative and angiostatic agent with minimal toxicity at pharmacological doses. However, the mechanism by which 2ME induces its anti-proliferative and apoptotic activities is uncertain, and its physiological function remains to be determined. In the present study we examined the effects of physiological and pharmacological concentrations 2ME in human skin microvascular endothelial cells.
Our findings show that 2ME rapidly activates JNK and p38 MAP kinase pathways at both physiological and supraphysiological levels. Our results indicate that the rapid activation of p38 and JNK are not required for 2ME-induced inhibition of DNA synthesis, caspase-3 activation, or cell cycle arrest, and thus apoptosis, in microvascular endothelial cells. On the contrary, our results suggest that rapid activation of JNK and p38 MAP kinases likely mediates an early pro-survival response to 2ME. The fine regulation of survival versus apoptotic signals may contribute to the ability of 2ME to elicit its effects in endothelial cells. Since endothelial cells play an essential role during wound healing, identification 2ME as a potent regulator of endothelial cell proliferation and function warrants further studies of its therapeutic potential as an agent to manipulate wound repair events.

This work was supported by a 'Canadian Institutes of Health Research' grant and a 'Heart and Stroke Foundation Quebec' grant to AP

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ANGIOGENESIS INDUCED BY ENRICHED BONE MARROW CELL TRANSPLANTATION IN ISCHEMIC HINDLIMB CANINE MODEL

Anabel S. de la Garza[1], Luis Padilla[1], Edgar Krötzsch[2], Siegfred A. Figueroa[1], Gerardo Avila[1], José A. Morales[3], German Glennie[1], Mauricio Di Silvio[1].
[1]Experimental Surgery Department, [2]Connective Tissue Laboratory, [3]Cell Culture Department, C.M.N. "20 de Noviembre", I.S.S.S.T.E. Mexico City, Mexico.

Bone marrow is an important supply for many progenitor cells, among them endothelial precursor cells. They have been already considered as potential therapeutic angiogenesis for ischemic hindlimb. In this work we performed an unilateral chronic ischemic hindlimb model in 20 dogs, we were dissected and ligated the middle sacra artery and external right iliac artery preserving the internal iliac artery, after one week we removed de femoral artery performing a proximal and distal ligation and inserted into the gracilis muscle 3 Silastic tubes (0.8 mm diameter) in a middle circle form in order to created fibrocollagenous tunnels. Fifteen days later, we obtained bone marrow (30 mL) and mononuclear cells were separated; at the same time, all tubes were removed and bone marrow cells were transplanted into the fibrocollagenous tunnels, only in the third and fourth groups. During the 8 at 12 days we administered subcutaneously saline solution to the first and third groups and G-CSF to the second and fourth groups. Finally, after 30 days we performed contrasted angiographies from both limbs and was calculated mean angiographic score (MAS) from the number of vascular intersections, also was taken a biopsy from the central portion of right gracilis muscle for vascular assessment, as well as for vascular proliferating cells by immunohistochemistry.
Results demonstrated that white bone marrow cell transplantation enrichment by G-CSF administration stimulates angiogenesis in ischemic hindlimb, twice than controls, and it is better than white bone marrow cell transplantation alone. White bone marrow cell is a potential therapy for ischemic hindlimb, because stem cells are growth factor provider, as well as an important source for endothelial precursors capable to form vessels *de novo*, mainly when this stem cells are deposited in an appropriate extracellular matrix, such as the fibrocollagenous tunnels in this model.

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A STUDY ON SCIENTIFIC VALIDATION OF THE INDIAN TRADITIONAL WOUND HEALING AGENT

A. Gnanamani[1], R. Gopal[1], Mary Babu[2] and S. Sadulla[1]
[1]CHORD, CLRI, Adyar, Chennai, India [2]Biomaterials, CLRI, Adyar, Chennai, India

Maththaan thailam, the *Datura* leaf juice is traditionally used for healing of wounds and related ailments in India. However, the scientific validity of the thailam has not been explored in the literatures. In the present study, the efficacy of maththaan thailam on healing of three types of wounds namely Open, Burn and Diabetic wound in animal models has been studied. Healing pattern and biochemical characterization of granulation tissue were assessed over the healing periods. Studies were more concentrated on to evaluate the change in the matrix metallo proteinase profile in the three different types of wound with respect to the applied thailam. Further supports were also expected from the antioxidant levels and histopathological examination of granulation tissue. It has been observed that among the three types of wounds, delayed healing was observed with diabetic wound. Complete healing of $2 \times 2\,cm^2$ wound size was achieved only after 22 days, whereas burn and open wound took only 16 and 9 days for complete closure. Further, MMP's profile also showed a variation in the expression of MMP2 and MMP9 with respect to healing property. Biochemical and histochemical results and the antioxidant levels are highly correlated with the healing property irrespective of the wounds

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ENGINEERING A HUMAN 'SKIN" WITH ADULT PRIMARY CELLS.

M. Martins-Green, QiJing Li, Min Yao.
University of California Riverside, Riverside CA, 92521.

The inability to experiment in humans creates a great need to develop culture systems that mimic human tissues/organs. Skin is arguably the simplest human tissue and therefore provides an excellent prototype for tissue engineering. Moreover, skin is the body's first line of defense.
Currently available skin replacements have been classified into four categories: (i) those that are composed completely of epidermal cells; (ii) those consisting of dermal components derived from processing of cadaver skin or from collagen and other matrix molecules; (iii) those containing both dermal and epidermal components and (iv) those that contain dermal, epidermal and vascular components. All of these substitutes have drawbacks. Therefore, there is a need for skin replacements that: (a) are prepared with pertinent primary human cells but yet can be ready "off" the shelf; (b) can be prepared rapidly; (c) contain stable structures in particular microvessels that can rapidly connect with the patient's vasculature in this manner establish circulation in the "graft" increasing the chances of survival; (d) can be tailored for specific wound impairments (e) are long lasting. We have developed a new generation human "skin" that can fulfil these requirements and can potentially be used as a "living bandage". We start with three primary human cell types and a collagen matrix that self-assemble into a connective tissue containing a network of mature microvessels, is covered with a stratified epidermis, expresses biochemical markers, matrix molecules, and cytokines characteristic of normal human skin and matures in 10–15 days. Moreover, two additional cell types, pericytes and monocytes, differentiate *in situ* adjacent to and within microvessels, respectively providing stability to the microvessels and the epidermis expresses keratins that are typical of mature skin and not those characteristically produced in response to injury such as keratins 6, 16, 17. This tissue can potentially be developed into a skin replacement for patients with impaired healing. In addition, this tissue responds normally to bological stimuli, providing a powerful vehicle to investigate mechanisms of skin development and regeneration, understand pathological processes, and test drugs and treatments for skin diseases

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BLOCKING IL-6 RECEPTORS INCREASES MATRIX METALLOPROTEINASE-3 PRODUCTION IN HYPERTROPHIC SCAR FIBROBLASTS

Mohan R.K.Dasu, Barrow RE, Hawkins HK, McCauley RL, Herndon DN
Shriners Childrens Hospital, Dept. of Surgery, UTMB Galveston, TX.

Introduction: We hypothesize that interleukin-6 receptor (IL-6r) blockade with IL-6r antibody will increase matrix metalloproteinase-3 (MMP-3) production in hypertrophic scar fibroblasts.
To test this hypothesis, MMP-3 protein levels were measured in fibroblasts cultured from normal skin and hypertrophic scar with and without IL-6 receptor blockade.
Methods: Primary cell cultures of normal skin (NSF) and hypertrophic scar fibroblasts (HTS) were obtained from the same patient ($n=5$) and seeded into 6 well plates ($40{,}000\,cells/cm^2$) in complete media. Cells were treated with and without IL-6 receptor antibody and IgG (10 ng/ml) for 6 hours. Protein concentrations of MMP-3 are determined in equal volume of supernatants by ELISA.
Statistical analysis was by unpaired *t-test* ($^*P<0.05$).
Results: Blocking IL-6 receptors with its antibody showed significant increase in MMP-3 protein concentration in HTS compared to NSF.

	MMP-3(ng/ml)		MMP-3(ng/ml)
NSF	1.6 ± 0.13	HTS	0.7 ± 0.37
NSF + IL-6r	0.3 ± 0.15	HTS + IL-6r	$2.3\pm0.5^*$
NSF + IgG	0.26 ± 0.16	HTS + IgG	0.75 ± 0.14

Conclusion: IL-6 receptor mediated signaling is involved in the pathogenesis of hypertrophic scar formation and its blockade may reduce hypertrophic scar formation.

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KINETIC EVALUATION OF SCAR AFTER FULL THICKNESS INCISION INJURY IN THE RAT

Kathleen E. Rodgers, Ph.D., Dolph D. Ellefson, Ph.D., Theresa Espinoza, M.D., Norma Roda, DVM, Sonia Maldonado, Gere S. diZerega, M.D.
University of Southern California KECK School of Medicine

Previous studies have shown that angiotensin peptides, $NorLeu^3$-A(1–7) in particular, accelerate dermal healing and reduce scar formation. In this report, the effect of this peptide on scar formation is more fully delineated. The effect of surgical day, time after injury and observer on the clinical appearance of the incision was determined. Clinical observations of incision site included inflammation, dehiscence of the injury and appearance of scar were conducted by two blinded observers (two observations per time point) twice weekly. The scores correlated between observers and for the same observer within an observation day. Dehiscence of the incision occurred in 35% to 40% of incisions early (days 4 and 7) after injury. Administration of $NorLeu^3$-A(1–7) at the time of injury reduced the incidence of dehiscence at day 7 to approximately 20%. Further, the length of the wound opening was significantly reduced in the peptide-treated incisions at day 7. Starting on day 14 after injury, scar formation was evaluated. Up to 80–90% of control animals had observable scars starting on day 14. Thereafter, the scar remodeled with fewer incisions having visible scar on day 28. With administration of $NorLeu^3$-A(1–7), significantly fewer incisions had observable scars starting on day 14 and throughout the study. As few as 20% of the incisions had observable scars on day 28. The histological appearance of the healing wound was also evaluated at weekly intervals starting on day 7 and continuing until day 42. At day 7, the maximal number of fibroblasts at the wound site was observed. Thereafter, the number gradually reduced, plateauing at day 28. The administration of peptide had no effect on fibroblast number at the incision site. A similar pattern was observed in the thickness of the epidermis with the resolution of te hyperplastic phase at day 21. Administration of the peptide significantly increased epidermal height at day 7. Blood vessel formation peaked on day 21 and 28 in control wounds and was further enhanced by peptide administration during the neovascularization phase. After day 28, blood vessel number was comparable between control and treated incisions. Collagen deposition and remodeling were are increased by the administration of $NorLeu^3$-A(1–7) at the time of injury. This paper describes the kinetics of scar formation in the rat by clinical and histological observation and further described the beneficial effect of $NorLeu^3$-A(1–7) on scar formation.
Essential Therapeutics, Waltham, MA