The Plastic Surgery Research Council

52\textsuperscript{nd} Annual Meeting

University of Stanford
Stanford, California

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No Photography or videotaping is allowed during the scientific sessions without prior approval by the Executive Committee.
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52ND ANNUAL MEETING OF THE
PLASTIC SURGERY RESEARCH COUNCIL

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WELCOME FROM THE CHAIRMAN

It gives me a great deal of pleasure to welcome you to the 52nd Annual Meeting of the Plastic Surgery Research Council hosted by the Division of Plastic and Reconstructive Surgery at Stanford University School of Medicine. The program offers the opportunity to enjoy a world-class scientific conference, in the beautiful setting of the San Francisco Bay Area. Dr. Michael Neu meister, the Scientific Program Chair, has prepared an exceptional program featuring concurrent sessions of state-of-the-art scientific research. Selecting the highest quality abstracts for this meeting, our Scientific Program Committee has exerted tremendous judgment and effort in ensuring the finest quality of Scientific Program. In addition, we have a unique aspect of this year’s program; there will be a panel including the President of the Orthopedic Research Society.

The Stanford University School of Medicine, Division of Plastic & Reconstructive Surgery has a long and rich tradition. Stanford Plastic Surgery was established in 1965 based on the vision of Dr. Robert A. Chase for integrated training in plastic surgery. This model is now widely accepted across the country. For over 40 years, Stanford has trained plastic surgeons, many of whom have contributed greatly to innovations in hand surgery, craniofacial surgery, microsurgery, aesthetic surgery, and overseas surgery. The Division’s current strengths are outstanding clinical care in all aspects of plastic and reconstructive surgery, a dynamic training program with 18 residents and 3 fellows, and 24 core faculty with diverse surgical and research interests. The faculty have received over 4 million dollars in annual extramural research funding for craniofacial biology, tissue engineering, wound healing, vascular biology, fetal wound healing, upper extremity biomechanics, virtual reality, and bioinnovation. The current mission of the Division is to translate this research into clinical innovations for plastic surgery.

The meeting format for this year begins on Wednesday evening, June 20, with the Welcome Reception. On Thursday, June 21, we will have the Local Program in the morning and the Scientific Session in the afternoon. That evening (Thursday), the Members Dinner will be held at the Thomas Fogarty Winery. On Friday, June 21, there will be morning and afternoon Scientific Sessions. Friday evening, is free and I encourage you to take advantage of the Bay Area restaurants and activities. Finally, on Saturday, June 21st, we will have a morning session followed by a luncheon, during which the awards will be announced for the winners of the five categories of best papers and posters.

It is truly an honor for me to host this 52nd Annual Meeting of the Plastic Surgery Research Council. This has really been a team effort and I want to acknowledge my colleagues who have assisted me every step of the way: Jim Chang, Geoff Gurtner, Peter Lorenz, and George Yang. I encourage everyone attending the meeting to enjoy the greater Bay Area following the meeting, including San Francisco, Lake Tahoe, Wine Country, Monterey/Carmel Peninsula, Yosemite National Park, and Gold Country, etc. I am looking forward to an outstanding scientific meeting and a thoroughly enjoyable four days here at Stanford.

Michael T. Longaker, MD, MBA
PSRC Chairman, 2007
Rod J. Rohrich, MD

This supplement to *Plastic and Reconstructive Surgery* represents a milestone not only for the Journal, but also for the American Society of Plastic Surgeons/Plastic Surgery Education Foundation and the Plastic Surgery Research Council (PSRC). For the first time, the official meeting abstracts of a sister society are published as a supplement to the Journal. In February of this year, did the Journal become an Official Organ of the PSRC, although strong ties between the Society and Council have existed for decades. Formalizing and finalizing the “official organ” status, and collaborating on this supplement, however, resulted from the efforts of numerous people within the Society and Council. Council members instrumental to this process were Greg Evans, Arun Gosain, Michael Longaker, James Bradley, Paul Cederna, Steve Buchman, Bill Kuzon, and Michael Longaker. ASPS/Plastic Surgery Educational Foundation leaders involved were past Society Presidents Bruce Cunningham and Scott Spear, past Foundation President Brian Kinney, Society Executive Vice President Paul Pomerantz, and Assistant Executive Director Lynn Kahn. As with every Journal endeavor, we could not have published this abstract without the collaboration and support of our publisher, Lippincott Williams & Wikins, represented by James R. Mulligan, publisher. PSRC Executive Director Catherine Foss and her dedicated staff, especially Jodie Ambrose, coordinated the receipt and review of the abstracts, and supplied expertly prepared documents and greatly facilitated the overall production of the supplement.

As mentioned in the Editorial in the February issue1, all subscribers to the Journal have access to all of the PSRC annual meeting abstracts. This is important because most of what eventually occurs scientifically in plastic surgery is presented in the Council’s abstracts 1 to 2 years in advance of it becoming mainstream with clinical plastic surgery.

The PSRC Meeting Abstract supplement to the Journal enjoys the benefits that all PRS supplements share:

- Full indexing in such abstracting services as Index Medicus, Abridged Index Medicus, Current Contents/Clinical Medicine, Life Sciences, Science Citation Index, Research Alert, ISI/Bio Med, EMBASE/ExcerptaMedica, and BIOSIS;
- Broad global readership through print and web subscriptions and online databases such as Ovid. Through the Ovid database, the Journal and its supplements have a potential readership of over 13 million end-users worldwide.
- Peer review of all contents. Abstracts will be reviewed by the PSRC, and only those accepted after undergoing review will be published.

Additionally, the contents of this supplement will be available through the PSRC website (www.psrc.org) as well as through the Journal’s website (www.PRSJournal.com).

An abstract represents the essence of a scientific study; the hard work of study conceptualization, data collection, and interpretation of results has been performed and is presented in these abstracts. The next step would be to submit a completed manuscript based on the abstract. We encourage all authors of abstracts to write up their findings and submit them as complete manuscripts to *Plastic and Reconstructive Surgery* through the online system, PRS’ Enkwell (www.editorialmanager.com/prs).

We all hope you enjoy this Abstract Supplement, and look forward to future joint ventures with the PSRC.

Rod J. Rohrich, MD
Editor-in-Chief
University of Texas Southwestern Medical Center
5909 Harry Hines Blvd., HD1.544
Dallas, TX 75235-8820
rjeditor_prs@plasticsurgery.org

References


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HISTORICAL PERSPECTIVE:
THE PLASTIC SURGERY RESEARCH COUNCIL

The following is compiled in part from
Peter Randall's Thirty-Five Year History of the
Plastic Surgery Research Council

Thomas S. Davis, MD
Historian Emeritus

In the early 1950’s there was a “feeling” among “younger” plastic surgeons (those recently boarded or not yet boarded) of a need for an arena to discuss research in plastic surgery, including works in progress. The major plastic surgery organizations were perceived as forums for politics and jousting fields for the political giants of the day. The American College of Surgeons initiated the Surgical Forum in 1949, and later the plastic surgeon section in 1953 under the direction of Dr. Joe Murray. Still, the impression among the younger plastic surgeons was that research was “taking a back seat” in the mainstream plastic surgery organizations.

An informal meeting was held in San Diego, California in 1954. The “young bucks” enlisted the advice of trusted and more senior advisors. Drs. Lewis T. Byars (St. Louis), Brad Cannon (Boston), and Truman G. Blocker (Galveston) listened and lent encouragement. Their recommendations were to hold these meetings in a university setting and to include the local university talent in discussions in the field of research. Caution was also given regarding the possible considerable opposition to the formation of such a group. It was agreed that they would meet the following year. Sixteen individuals were picked to be invited to an “Organizational Meeting” held in Baltimore in the fall of 1955. The word “picked” implies selecting a few from the many, whereas actually this was more a search for the “any” from the “few.”

At the time of the founding meeting of the Plastic Surgery Research Council in 1955 at Johns Hopkins, called by Drs. Milton Edgerton and Robin Anderson, Dr. Richard Stark submitted the drawing of Baronio’s Sheep (1804) with auto and allografts in situ as a possible logo for the council.

Searching for a form other than the logo cliche of the circle, the design was made into a rectangle with rounded corners. It was accepted and has been the logo of the council since that time.

Following this first official meeting, Dr. Robin Anderson was asked to compose a Constitution and a set of By-Laws. A major objective of this Constitution and By-Laws was to maintain “a pure and virtuous” society “by avoiding the hierarchic pitfalls and elitist attitudes of the established societies.” The members also decided not to align this meeting with any other plastic surgery organization. This two-page document was approved at the second meeting (1956) in Jackson, Mississippi, hosted by Dr. Jim Hendrix. The name of the organization was debated. One suggested the name, “Plastic University Surgeons” (“P.U.S.”). However, the word “University” itself implied restriction and was ultimately discarded. The word “Council” was eventually (but not initially) chosen, and had literally been taken from the Indian usage of the word to indicate an open gathering of all those concerned. The name of the organization, “The Plastic Surgery Research Council,” was officially adopted at this meeting. It has served this purpose well.
In 1981, The Peter Gingrass, MD Memorial Award was established by Dr. Ruedi Gingrass on behalf and in memory of his brother Peter. The award recognizes the medical student or non-plastic surgery resident presenting the best paper at the annual meeting of The Plastic Surgery Research Council. In 1982, The John F. Crikelair, MD Research Award was funded by George Crikelair in memory of his son to recognize the best paper at The Plastic Surgery Research Council by a high school or college student. The Clifford C. Snyder, MD Past-Chairman Award was established in 1983 by Clifford Synder and funded in part by various past chairmen to recognize the best paper presented by a Plastic Surgery Resident or Fellow at the Plastic Surgery Research Council annual meeting. In 1997, The Best Poster Award was established by Dr. Robert Hardesty to recognize the value and contributions of Poster Presentations at the annual meeting of the Plastic Surgery Research Council. In 2000, the Shenaq International Research Award for the best paper by a foreign medical school graduate was funded by Dr. Sal Shenaq.

As time passed, the Research Council not only grew in numbers but also in respect among the “other societies and associations.” The Research Council was formally asked to elect representatives to the American Association of Medical Colleges, Council of Academic Societies, Plastic Surgery Academic Advisory Council, American College of Surgeons (Plastic and Maxillofacial Council), American Association of Plastic Surgeons, the Plastic Surgery Educational Foundation Research Grants Committee, and the Council of Plastic Surgery Organizations. We are also asked to nominate candidates for the American Board of Plastic Surgery each year.

It is interesting to note that the basic tenants set down in the formative years of the Council have been maintained as guidelines for The Plastic Surgery Research Council. The original goals and concepts of an open forum with free discussion of work completed and work in progress continue, and the suggestions of “keep it young” and meet independently at research institutions continue. Specifically, at age 50 active members become senior members (with corresponding loss of voting privileges and inability to hold elected office) assuring a youthful and progressive leadership.

“Of the sixteen founding members of the Plastic Surgery Research Council, five have become Presidents of the American Society of Plastic and Reconstructive Surgeons (ASPS); four have become Presidents of the American Association of Plastic Surgery (AAPS); thirteen have been training program directors; three have been ‘Clinician of the Year’ of the AAPS; and two have received a Special Achievement Award of the ASPS. Eleven have become Directors of the American Board of Plastic Surgery; four have been Chairmen of the Board; and three have been Vice-Chairman of the Board”; one received the Nobel Prize in Medicine for his research on transplantation. What a magnificent heritage.

The Baronio Sheep

From time to time, members and others ask the origin of the sheep drawing that is the PSRC’s official logo. The following attestation sheds a little light on this subject:

Degli innesti animali. Milan: Dalla Stamperia e Fonderia del Genio, 1804. This landmark work in the history of plastic surgery details the results of Baronio’s experiments on autogenous skin grafting in animals at the close of the eighteenth century, the first such study to be carried out in scientifically organized experiments. Baronio (1759-1811), physician and naturalist, records and illustrates the first demonstration of a successful graft, removing skin from a sheep and transplanting it to a new site on the same sheep within an hour. The technique was successfully applied in humans some thirteen years later.
Thursday, June 21, 2007

Session 1A
Fetal Surgery/Wound Healing
Abstracts 1A – 5A
4:00 – 4:40 pm

Papers are reprinted as they were submitted.
The PSRC takes no responsibility for typographical or other errors.
**1A**

**WNT SIGNAL IS CRUCIAL FOR SKIN WOUND REGENERATION**

**Presenter:** Joseph A. Knowles, BS  
**Authors:** Knowles JA, Hantash BM, Longaker MT, Lorenz HP  
Stanford University

**Introduction:** Wound healing differs substantially between fetal and adult tissue. Wounded adult skin heals with scar formation and is devoid of adnexal structures. Fetal skin heals scarlessly and restores adnexal structures. Previous studies have shown beta-catenin (Wnt) signaling plays an important role in hair follicle formation, epidermal proliferation and stem cell migration. We therefore examined the role of the Wnt signaling pathway in healing fetal skin.

**Methods:** TOPgal Wnt reporter mice express beta-galactosidase (beta-gal) in response to Wnt signaling. Fetal skin (E16.5) was transplanted onto the chorioallantoic membrane of a developing chick embryo to create an ex utero, in ovo model for fetal wound healing. Circular wounds of 1 mm diameter were created 48 hours post-transplant. To repress Wnt signaling, wounds were infected with adenovirus containing a gene for Dickopf-1 protein (Adeno-Dkk1), a protein antagonist that competes for the Wnt co-receptor Lrp. Skin wounds were stained for beta-gal on days 1-7, and analyzed histologically for Wnt activity. Follicular regeneration was assessed using immunohistochemistry and wound size was measured by digital photography.

**Results:** Fetal wounds treated with Adeno-Dkk1 showed decreased beta-gal staining. H&E staining demonstrated reduced keratinocyte proliferation and migration at the wound edge in Adeno-Dkk1-treated skin grafts on days 1 to 4. Histological analysis revealed regeneration of follicular structures and collagen deposition at day 7 was also diminished in adenovirus treated wounds compared to controls.

**Conclusions:** We successfully demonstrated blockade of Wnt signaling by infecting transplanted fetal skin grafts with adenovirus carrying the Wnt antagonist Dkk1. Interference with Wnt signaling post-wounding led to impairment of follicular regeneration and collagen deposition. Our data also revealed a reduction in keratinocyte proliferation and migration upon blockade of Wnt signaling. Taken together, our data suggest that Wnt signaling plays an important role in fetal wound healing by contributing to both epidermal and dermal regeneration.

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**2A**

**THE ROLE OF MESENCHYMAL STEM CELLS (MSCS) IN EPC-MEDIATED ISCHEMIC VASCULOGENESIS IN DIABETES**

**Presenter:** Matthew J. Callaghan, MD  
**Authors:** Callaghan MJ, Grogan RH, Chang E, Gurtner GC  
Stanford University

It is known that bone marrow progenitor cells contribute to ischemic neovascularization and that this process is impaired in diabetes. However, characterization of these cells and their specific dysfunction in diabetes remains unclear. Here we identify MSCs as the progenitor subset mobilizing from the bone marrow to engraft in peripheral tissue in response to ischemia and show an impairment in diabetes at the mobilization step.

A model of soft tissue ischemia was utilized in diabetic mice. Bone marrow, whole blood and ischemic skin were subject to FACs analysis at timepoints from D0 - D14. Bone marrow MSCs were identified as lin-/sca1+/CD45-. Hematopoietic cells (HSCs) were identified as lin-/sca1+/ckit+/CD45+. In circulation, EPCs were identified as lin-/flk1+/CXCR4+. To identify progenitor cells engrafted in peripheral tissue, a parabiotic model was employed pairing GFP and wild-type mice. Two weeks after surgery on the wild-type mouse, tissue was harvested and analyzed by FACs for endothelial and bone marrow markers. Vascular density and progenitor engraftment was confirmed by CD31 and GFP immunohistochemistry.

FACS analysis revealed a significant decrease in bone marrow MSCs immediately after surgery. This population was restored by D7 in wild-type but not diabetic mice. Circulating EPCs were depleted 12hrs after surgery in diabetes but not wild-type mice. Engrafted bone marrow-derived cells were CD45-, indicating an MSC origin. Vascular density was significantly reduced in diabetic animals, evidenced by fewer CD31+/flk1+ cells by FACs analysis and lower vessel counts by CD31 immunostaining.

This study represents a significant step in identifying the origin of EPCs and describing their vasculogenic impairment in diabetes. For the first time, we are able to confirm a mesenchymal precursor cell gives rise to circulating EPCs. Furthermore, this process was found to be arrested at the mobilization step in diabetes. Augmenting the MSC population by cell-based therapies or mobilizing agents represent powerful alternatives to current therapies for the ischemic vascular complications of diabetes.
3A
TARGETED GENE THERAPY FOR ISCHEMIC TISSUE VIA VIRAL TRANSFECTION OF AN EXPRESSION CASSETTE CONTAINING MULTIPLE REPEATS OF HYPOXIA RESPONSE ELEMENT

Presenter: Kevin J. Cross, MD
Authors: Cross KJ, Bomsztyk ED, Weinstein AL, Spector JA, Lyden DC
Weill Medical College of Cornell University

Introduction: HIF-1alpha is a transcription factor that is rapidly degraded under normoxic conditions but stabilized during periods of ischemia. HIF-1alpha binds to the enhancer HRE and drives downstream gene expression. We hypothesized that a viral vector containing multiple repeats of HRE can preferentially upregulate expression of specifically targeted genes in cells cultured under hypoxic conditions compared to normoxic controls.

Method: We created a custom designed expression cassette containing 9 repeats of HRE linked to the reporter gene beta-galactosidase and inserted it in an AAV vector. HEK cells were plated and grown in normoxic (21% O2) chambers (1 million cells/plate). They were then transfected with 10,000 pu AAV-H9-LacZ/cell. After 24 hours, half of the plates were placed in a hypoxic (1% O2) culture chamber. All cells were harvested 1 day later. The activity of LacZ was measured by conversion of galactoside red-ß-D-galactopyranoside to galactose and phenol red, as measured by a spectrophotometer. The experiment was performed 4 times, each in triplicate. Results are expressed an average of the 4 experiments.

Results: Transfected cells exposed to normoxic conditions demonstrated 0.496(+-0.068)U/µg. Transfected cells exposed to hypoxic conditions for twenty-four hours demonstrated LacZ activity of 2.9(+-0.58)U/µg. LacZ activity of non-transfected cells was 0.28U/µg. Thus, transfected cells exposed to 24 hours of hypoxia show greater than a 10-fold increase in LacZ activity compared to baseline normoxic controls.

Discussion: We have proven that viral delivery of a custom designed expression cassette containing HRE linked to a gene of interest will cause upregulation of the target gene during periods of ischemia but not during physiologic normoxia. This is the first step towards our goal of preventing ischemia-reperfusion (IR) related injury in selected tissues via targeted, ischemia-specific gene therapy. By driving the expression of “protective” genes such as hemoxygenase during periods of ischemia, we hope to ameliorate the devastating effects of reperfusion injury.

4A
VASCULARIZATION DURING TISSUE INJURY REPAIR IS ENHANCED BY TOPICAL TREATMENT WITH ADIPOSE-DERIVED STROMAL CELLS

Presenter: Matthew W. Blanton, MD
Authors: Blanton MW, Johnstone BH, Hadad I, Mund JA, Rogers PI, March KL, Eppley BL
Indiana University

Background: We tested whether promotion of wound healing could be enhanced by incorporating autologous, pluripotent adipose-derived stromal cells (ASC) into fibrin-based matrices.

Methods: A full-thickness porcine incisional wound model (n=132 wounds) was used to compare wound healing properties of 6 topical treatments: ASC+platelet rich plasma (PRP); PRP alone; platelet-poor plasma (PPP) alone; ASC+PPP; allogeneic ASC containing a transgene for green fluorescent protein (GFP-ASC)+PPP and saline (control). Autologous peripheral blood was processed using a GPS device. The PRP and PPP fractions (with and without admixed ASCs) were activated and sprayed onto the wounds and dressed. Wounds were monitored for 3 weeks by visual inspection and biopsies on days 3, 7, 14 and 21. Each biopsy was analyzed using histology and immunohistochemistry to detect arterioles, myofibroblasts and GFP-ASC. Treatment preparations were analyzed in vitro for VEGF, PDGF-BB and TGF-ß1 concentration using ELISA.

Results: No difference was observed in the wound contraction rate for any of the treatments used in juvenile, healthy pigs. However, quantitative analyses of vessel ingrowth and myofibroblast proliferation in newly formed dermis indicated treatments with ASCs lead to improved wound healing compared to all other treatment groups (p<0.05). This improvement correlated with 7x higher proangiogenic VEGF levels in matrices containing ASCs compared to PRP or PPP (p<0.05). To determine if the increased vascularity of healed wounds could be attributed to incorporation of differentiated cells into neovasculature, an analysis of newly formed dermis in transgenic GFP-ASC indicated many ASCs survived to 3 weeks. Furthermore, ASCs predominantly localized to perivascular regions.

Conclusion: We describe an improved wound healing treatment based on a combination of autologous, pluripotent cells and platelet rich matrix. The enhancement over PRP alone is partially explained by the locally confined augmentation of factors, especially VEGF. The localization of ASCs to the repaired dermis may indicate a direct physical support to vessel growth.
AGING IMPAIRS INTERLEUKIN 1–BETA INDUCED STROMAL DERIVED FACTOR-1 ALPHA SIGNALING THROUGH DECREASED LEVELS OF HYPOXIA INDUCIBLE FACTOR-1 ALPHA

Presenter: Shang A. Loh, MD
Authors: Loh SA, Lin D, Chang EI, Gurtner GC
Stanford University

Introduction: Impaired wound healing in the elderly is clinically well documented. IL1-beta plays a central role in the inflammatory response by recruiting other inflammatory cells and stimulating production of downstream cytokines, such as SDF-1. We have previously shown that the SDF-1 promoter contains hypoxia response elements (HRE). IL1-beta has been shown to utilize HIF-1 to affect transcription of downstream targets. Here we investigate the role of age on IL-1beta stimulated SDF-1 alpha upregulation through the HIF-1 pathway.

Methods: Two full thickness 10 mm circular wounds were made on the dorsum of young and aged mice, which were harvested at days 0, 1, 3, 5, and 7. Wound cross sections were stained with H&E. Protein and RNA were harvested from wound samples and primary aged and young cultured murine fibroblasts with and without IL1-beta stimulation. Real-time PCR and Western blots were performed to evaluate SDF-1 alpha and HIF-1 alpha levels. Primary cells were also transfected with luciferase reporter constructs containing either wild-type murine SDF-1 promoter or a mutated promoter with nonfunctional HRE sites. After 18-24 hours, the cells were stimulated with 30 pg/ml of human IL1-beta and luciferase activity assayed.

Results: Young H&E sections demonstrated a trend toward a slightly narrower epithelial gap compared to the aged at day 7. Wound SDF-1 levels peaked at day 1 and returned to baseline by day 3. Both showed similar fold increases in SDF-1 on day 1; however young mice had higher SDF-1 levels at all time points. IL1-beta stimulated aged primary fibroblasts demonstrated an impaired ability to upregulate SDF-1 expression compared to young. Western blots showed decreased baseline levels of HIF-1 alpha in aged fibroblasts. Aged fibroblasts exhibited an impaired SDF-1 luciferase response to IL1-beta stimulation compared to young. Transfection with the mutated SDF-1 reporter negated the effects of IL1-beta.

Conclusions: Higher baseline SDF-1 levels may help promote healing in younger mice. Impaired ability of aged fibroblasts to upregulate SDF-1 may be due to decreased levels of HIF-1 alpha.
Thursday, June 21, 2007

Session 2A
Nerve
Abstracts 6A – 11A
5:00 – 5:50 pm

Papers are reprinted as they were submitted.
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6A

AUTOGENOUS AND ALLOGENIC SCHWANN CELLS IN NERVE ALLOGRAFTS INDICATE THAT ANTI-CD40 LIGAND COSTIMULATORY BLOCKADE INDUCES A PERMISSIVE STATE

Presenter: Paul S. Cederna, MD
Authors: Cederna PS, Yu D, Urbanchek MG
University of Michigan

Introduction: Schwann cells (SC) provide significant antigenic stimulation and as a result, define the fate of SC in nerve allografts will help to define the duration of antigenic stimulation following allotransplantation. We hypothesize that following nerve allografting, SC ultimately are rejected and replaced with autogenous SC from the proximal and distal nerve ends. Following CD40/CD40L blockade, the nerve allograft becomes a hybrid containing a mixed population of allograft and autogenous SC. In addition, this co-stimulatory pathway blockade leads to improved end organ reinnervation as compared to nerve allografting alone.

Methods: Rosa26Bl/6 mice are a C57Bl/6 mutant strain containing the beta-galactosidase gene that stains SC an insoluble blue. We studied four groups of Rosa26Bl/6 mice (n = 6); three groups received an 8 mm sciatic nerve graft while the Sham group had no graft. The Allograft with saline and the Allograft with CD40/CD40L mAb (MR1) groups each received BALB/c nerve grafts while the Isograft group received C57BL/6 grafts. MR1 was administered IP on day 0 to the allograft with MR1 group while the other groups received saline. On POD 21, extensor digitorum longus (EDL) muscles were harvested and stained by X-gal assay for beta-galactosidase.

Results: Sham Rosa26Bl/6 nerves contained abundant β-Gal+ SC throughout the nerve and isogeneic C57BL/6 nerve grafts demonstrated only β-Gal-SC. Nerve allotransplantation with MR1 demonstrated a mixture of β-Gal+ Rosa26Bl/6 and β-Gal-C57BL/6 SC indicating that some autogenous SC migrated into the nerve graft while some allograft SC remained. Nerve allotransplantation without MR1 demonstrated primarily β-Gal- C57BL/6 autogenous SC with a much smaller percentage of β-Gal+ Rosa26Bl/6 SC within the nerve graft. Axons in the allograft with saline group demonstrated acute rejection with very few β-Gal+Rosa26Bl/6 SC present. Boxplot 1 demonstrates the recovery of EDL force in all experimental groups; sham > allograft with MR1 > allograft. It also demonstrates that nerve allografts may not be completely replaced with autogenous SC but in fact contain a mixture of autogenous and allogenic SC when MR1 immunomodulation is utilized at the time of allotransplantation.

Conclusions: This work emphasizes the potential promise of CD40/CD40L blockade in reducing nerve allograft rejection and permitting meaningful muscle reinnervation. It also demonstrates that nerve allografts may not be completely replaced with autogenous SC but in fact contain a mixture of autogenous and allogenic SC when MR1 immunomodulation is utilized at the time of allotransplantation.

7A

TRANSPLANTATION OF EMBRYONIC STEM CELL DERIVED MOTOR NEURONS INTO DENERVATED MUSCLE ENHANCES MOTOR FUNCTIONAL RECOVERY AFTER NERVE INJURY

Presenter: Tateki Kubo, MD, PhD
Authors: Kubo T, Randolph MA, Winograd JM
Massachusetts General Hospital-Harvard Medical School

Introduction: We previously reported transplantation of embryonic stem (ES) cell derived motor neurons (MNs) enhanced muscle preservation for 7 days after denervation through the formation of new neuromuscular junctions (NMJ). In the current study, we performed transplantation in the setting of a nerve repair to determine the effect of transplantation of these cells on the functional outcome following repair. We also performed delayed transplantations into denervated muscle to attempt a rescue of muscle following denervation atrophy.

Methods: Murine GFP/HB9 ES cells were differentiated into MNs. To assess the effect on functional recovery, tibial nerves of nude mice were transected and underwent repair following transplantation of MNs into gastrocnemius muscles. Control animals received PBS injections. Functional recovery following nerve repair was evaluated with walking track analysis. In the rescue experiments, tibial nerve transection was performed without nerve repair, and MNs were transplanted into the nude mouse gastrocnemius muscle immediately after transection or 3 weeks after denervation. Quantitative and histological assessments of gastrocnemius muscle were done at days 7 and 21 after transplantation.

Results: GFP/HB9 ES cells were differentiated into GFP+ fluorescent MNs. Functional recovery after nerve repair with MNs transplantation by walking track analysis was significantly enhanced compared to PBS injected control group. In the rescue experiment of tibial nerve transection without nerve repair, the gastrocnemius muscles injected with MNs immediately after nerve transection were less atrophied than control PBS injected muscle at both days 7 and 21, while the muscles injected 3 weeks after denervation were not preserved. Co-cultures of MNs and myotubes formed NMJs in vitro, confirmed by the synaptic markers, as observed previously in vivo.

Conclusion: Following tibial nerve repair, MN transplantation improved motor functional recovery. Transplantation of MNs prevented muscle atrophy following denervation but was not capable of rescuing denervated muscle once atrophy had occurred.
8A REAL TIME IN VIVO IMAGING OF NEURAL MICROARCHITECTURE INCLUDING INDIVIDUAL AXONS AND MYELINATION WITH COHERENT ANTI-STOKES RAMAN SCATTERING (CARS) MICROSCOPY

Presenter: Francis P. Henry, MD
Authors: Henry FP, Coté D, Randolph MA, Kochevar IE, Lin CP, Winograd JM
Massachusetts General Hospital, Harvard Medical School

**Introduction:** Current analysis of nerve injury and repair relies largely on electrophysiological and ex vivo histological techniques. In vivo architectural assessment of nerve without removal or distruption of the tissue would greatly assist in the grading of neural injury and in the monitoring of regeneration over time.

CARS Microscopy is a nonlinear optical process using ultrashort laser pulses to probe molecular vibrational structures and conformations in tissue with a particular sensitivity for high lipid-containing molecules such as myelin. This minimally invasive, non-thermal technique offers high resolution images of neural microarchitecture, which we aim to evaluate in both normal and injured nerve.

**Methods:** A standard demyelinating crush injury was reproduced in the sciatic nerves of male Sprague Dawley rats. Animals were randomized into groups and CARS microscopy was undertaken at day 1 and weeks 2, 3 and 4 following injury. The uninjured nerve was used as a control. Functional analysis was undertaken weekly with standardized walking track analysis. Histomorphometry of both control and injured nerve was undertaken following imaging to allow verification of our findings.

**Results:** All animals demonstrated loss of sciatic nerve function following nerve injury. Recovery was documented with sciatic functional index data approaching normal at three weeks. Demyelination was confirmed in nerves up to three weeks post injury. Remyelination was observed in the three week group and beyond. Imaging of the control nerves revealed structured myelin bundles. These results were consistent with histological findings.

**Conclusions:** We conclude that CARS Microscopy has the ability to image the peripheral nerve following demyelinating crush injury. This technology which permits in vivo, real time microscopy of nerves at a resolution of 5-10 microns could provide invaluable diagnostic and prognostic information about intraneural preservation and recovery following injury.

9A IN VIVO REGULABLE VEGF165 OVEREXPRESSION BY EX VIVO EXPANDED KERATINOCYTE CELL CULTURES PROMOTES NEO-VASCULARISATION AND HEALING IN PORCINE FULL THICKNESS WOUNDS

Presenter: Stijn Dickens, MS
Authors: Dickens S, Vermeulen P, Van den Berge S, Hendrickx B, Vranckx JJ, KU Leuven
University Hospitals

**Introduction:** Ex vivo gene transfer of growth factors shows great promise for gene delivery into tissue in a pathological state. Vascular Endothelial Growth Factor 165 (VEGF) promotes attraction of endothelial cells and induces vascularisation of granulation tissue in wounds. Therefore VEGF seems a promising proangiogenic candidate for gene therapy in full thickness skin wounds (FTW) or 3D-tissue engineering constructs. Regulation of VEGF after gene therapy is mandatory to prevent potential hazardous side effects of overexpression and offers the opportunity to provide sequential overexpression.

**Methods:** VEGF was ligated in a tetracycline (TC)-inducible expression plasmid. We cotransfected this plasmid with a TC-repressor-plasmid into porcine keratinocytes (KC) using a liposomal-mediated transfection protocol. In vitro, a dose-response curve was established by daily adding 0 to 5 mug/ml TC to the medium. We analyzed VEGF expression in cell supernatant. In vivo, FTW were treated with VEGF-transfected KC (0 or 1 mug/ml TC) versus saline controls in a standardized porcine wound model. Wound fluid was collected daily and examined by ELISA. Biopsies from day 8 and 10 were evaluated by H&E and immunohistology staining with the lectin BS-1, CD144 and fibronectin.

**Results:** Highest regulable in vitro VEGF expression (28600 pg/ml) was obtained by 1 mug/ml TC. In vivo, we obtained significant upregulated levels of VEGF when 1 mug/ml TC was directly added to FTW as compared to controls. Immunostaining showed increased neo-vascularisation (p<0.001) and faster reepithelialization (p<0.01) in TC triggered wounds. Fibronectin deposition in the dermal matrix (p<0.01) was increased in KC treated wounds compared to controls.

**Conclusion:** We obtained regulable VEGF expression in vitro and in vivo and found 1 mug/ml to be the optimal TC concentration to trigger VEGF expression. This upregulation leads to enhanced neo-vascularisation, matrix deposition and faster reepithelialization in a porcine FTW model. We currently use this platform for regulable ex vivo gene transfer to enhance angiogenesis in tissue engineered constructs.
10A
LIPOREMODELING THROUGH
MANIPULATION OF THE NPY2 RECEPTOR
Presenter: Stephen B. Baker, MD, DDS
Authors: Baker SB, Kuo L, Johnson M, Zukowska Z
Georgetown University

Purpose: The neuropeptide Y2 receptor is proadipogenic when stimulated and adipolytic when blocked. This study evaluates the effect of NPY antagonists to eliminate peripheral body fat and the effect of NPY2 agonists to stimulate fat grafts in multiple murine models.

Methods: Different types of obese mouse models were injected subcutaneously with NPY2 antagonist. 3-D MRI was used to quantify loss of peripheral body fat. The following tests were performed: serum cholesterol, glucose tolerance, and insulin resistance. The liver, kidney, muscle, bone, and brain underwent histologic evaluation. Cr clearance and LFTs were also performed.

NPY2 agonist was injected into wild type mice and nude mice with grafted human fat. Human fat was grafted to nude mice and augmented with NPY2 agonist. Grafted fat was assessed at intervals with ultrasound, histology, and 3-D MRI. Final assessment was at 3 months. Student’s T test and ANOVA showed significance.

Results: NPY2 antagonist resulted in 45% reduction of peripheral body fat in ob/ob mice and 50% reduction in the high fat/stressed mice. Serum cholesterol and glucose tolerance were improved, and there was no change in insulin resistance in the NPY2 antagonist group. No adverse effects were found on examination of the kidney, liver, muscle, bone, or brain with the exception of a slight increase in bone density. Administration of NPY agonist showed increased graft retention. PECAM staining revealed increased angiogenesis and ultrasound showed less vacuolization of fat.

Conclusions: Injection of NPY2 antagonist results in loss of peripheral fat volume and the agonist enhances fat graft volume.

11A
TRANSPLANTATION OF HUMAN KERATINOCYTES INTO INJURED PERIPHERAL NERVE ELEVATES NGF LEVELS AND INDUCES HYPEREXCITABILITY OF SENSORY NEURONS
Presenter: Christine Radtke, MD, PhD
Authors: Radtke C, Vogt PM, Kocsis JD
Hannover Medical School; Yale School of Medicine

Introduction: NGF is produced by skin keratinocytes (KT) and hypersecretion of NGF has been suggested to contribute to changes in peripheral sensory neurons leading to hyperalgesia following skin inflammation. The present study was performed to investigate the effect of human KTs (hKT) transplanted into injured peripheral nerve on sensory neuronal excitability.

Methods: hKTs were microinjected into the proximal end of ligated nude rat sciatic nerve. Two weeks later the dorsal root ganglia (DRG) were removed, dissociated and prepared for in vitro whole cell patch clamp recording. Membrane and firing properties were studied from medium-sized (34-43 micron diam.) neurons in current clamp mode. The nerves were assayed for NGF using ELISA. The hKTs were labeled with a vital dye (PKH26) prior to transplantation.

Results: The transplanted hKTs survived and integrated into the ligated sciatic nerve. In recordings from control DRG neurons step depolarizations (0.1 to 1.0 nA; 100 msec. duration) typically resulted in a single action potential near the onset of the depolarization. In the sham control group (nerve ligation with DMEM injection) 14% of neurons gave rise to multiple spike discharge. In the hKT transplant group 85% of the neurons gave rise to multiple spike discharge to a single step depolarization and a large number showed membrane oscillations from which spontaneous spikes were generated. ELISA indicated high levels of NGF in the engrafted nerves. The transplanted rats exhibited pain symptoms.

Conclusions: Our findings indicate that transplantation of hKTs into injured peripheral nerve results in a substantial increase in nerve NGF and profound hyperexcitability of DRG neurons. Spontaneous membrane oscillations often gave rise to burst firing. Thus, hyperexcitability associated with elevated KT-derived NGF levels may alter DRG ion channel composition conducive to hyperexcitability. These data indicate that cellular release of NGF by KT have a profound effect on sensory neuronal excitability, and have implications for neuropathic pain mechanisms in hyperproliferative KT states such as after burn injury.
Friday, June 22, 2007

Session 3A
Limb Transplant
Abstracts 12A – 20A
7:00 – 8:20 am

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**12A**

**PROLONGED SURVIVAL OF VASCULARIZED SKIN ALLOGRAFTS ACROSS A FULL MHC MISMATCH USING ALLOANTIGEN-PULSED GM+RAPA DENDRITIC CELLS**

**Presenter:** Elaine K. Horibe, MD  
**Authors:** Horibe EK, Sacks JM, Unadkat JV, Ikeguchi R, Marsteller D, Thomson AW, Lee WP, Feili-Hariri M  
**University of Pittsburgh**

**Introduction:** Prevention of acute skin rejection in composite tissue allograft (CTA) transplantation can be better studied in vascularized skin allografts, however, literature lacks reports on long-term acceptance of vascularized skin allografts without continuous immunosuppression. This study compared tolerogenic properties of different host bone-marrow (BM)-derived dendritic cells (DC) populations and their potential to induce acceptance of vascularized skin allograft in rats across a full MHC mismatch with transient immunosuppression.

**Methods:** DC were generated either with GM-CSF (GM)+rapamycin and pulsed with donor alloantigen (Ag) (GM+Rapa DCp) or GM+IL-4 with or without alloAg (GM+IL-4 DCp or GM+IL-4 DC). DC phenotype and function were assessed by FACS, ELISA and MLR. Forty-five epigastric vascularized skin allografts were transplanted in 7 groups: I) untreated, II) cyclosporine (CsA, 10mg/Kg, day 0-20), III) CsA+anti-lymphocyte serum (ALS, day -4,+1), IV) +GM+IL-4 DC (days +7,+14), V) +GM+IL-4 DCp, VI) +GM+Rapa DCp and VII) +donor BM. Recipients’ T cell responses, graft appearance/histology and skin graft challenges were assessed postoperatively.

**Results:** Both DCs were myeloid with an immature phenotype, showed low T cell stimulatory capacity and produced minimal pro-inflammatory cytokines upon activation. Significant prolongation of allograft survival (>180 days) was observed in 3/6 rats in group VI (p<0.05). In group VII, 2/6 died of GVHD. T cells from groups V and VI were less responsive to donor Ag on POD30, but reacted to third-party Ag. Long-term surviving vascularized skin allografts in group VI showed preserved architecture on POD150 and upon skin graft challenge, these recipients rejected third-party skin grafts faster than the donor graft.

**Conclusion:** DC populations showed tolerogenic profiles in vitro. GM+RapaDCp combined with transient immunosuppression prolonged allograft survival. While this protocol avoided GVHD, the administration of DC postoperatively rendered it clinically applicable, since CTA are obtained from non-living donors. Studies on mechanisms involved are currently underway.

**13A**

**FGF2 REGULATES SELF-RENEWAL OF MOUSE ADIPOSE- AND BONE MARROW-DERIVED MESENCHYMAL STEM CELLS**

**Presenter:** Longmei Zhao, MD, PhD  
**Authors:** Zhao L, Li S, Shi Y, Hantash BM  
**Stanford University**

**Introduction:** Mesenchymal stem cells (MSCs) derived from adipose (AMSCs) or bone marrow (BMSCs) represent important reservoirs for regeneration of mesodermal tissues such as bone, cartilage, adipose, muscle and tendon. However, realization of their full therapeutic potential requires a detailed understanding of the mechanism by which MSCs self-renew. Recent studies have demonstrated that basic fibroblast growth factor (FGF2) regulates self-renewal of embryonic stem cells (ESCs). The present study was performed to determine whether a similar regulatory pathway controls MSC self-renewal.

**Methods:** BMSCs and AMSCs were freshly isolated from mice and cultured in the appropriate media. At passage 8, MSCs were plated in either a low or high serum media containing 0 or 10 ng/ml FGF2 for 3 or 14 days, respectively. Self-renewal and lineage specific marker expression was examined by RT-PCR. Multipotency was assessed using histochemical and immunocytochemical staining.

**Results:** After 3 days in low serum medium, expression of self-renewal markers Oct-4, Stella, Nanos3, and Abcg2 mRNA was increased up to 3-fold in BMSCs and AMSCs treated with FGF2 relative to controls. After 14 days in high serum medium, Oct-4, Stella, Nanos3, and Abcg2 mRNA expression was enhanced up to 6-fold in BMSCs and AMSCs exposed to FGF2 versus no treatment. Both cell types stained positively with anti-Sca-1 antibody after 3 or 14 days in culture. BMSCs maintained a greater propensity for osteogenic and adipogenic differentiation compared to AMSCs, as evidenced by increased alizarin red and Adipo-Red positive staining, respectively.

**Conclusion:** Our results demonstrate that self-renewal of MSCs is under the control of FGF2 and involves upregulation of Oct-4, Stella, Nanos3, and Abcg2 transcription. FGF2 did not alter purported self-renewal markers, such as Bmi-1, suggesting a specific rather than generalized transcriptional regulation pattern. In conclusion, adult MSCs and ESCs may employ strikingly similar mechanisms of self-renewal.
14A

RAPAMYCIN CONDITIONED DONOR ANTIGEN PULSED DC INDUCE LONG TERM HIND LIMB ALLOGRAFT SURVIVAL

**Presenter:** Jignesh V. Unadkat, MD  
**Authors:** Unadkat JV, Ikeguchi R, Sacks JM, Horibe EH, Thomson AW, Lee WP, Feili-Hariri M  
**University of Pittsburgh Medical Center**

**Introduction:** Need for chronic immunosuppression precludes the use of composite tissue allograft (CTA) as a routine surgical reconstructive option. Benefits of CTA are belittled by the complications of chronic immunosuppression such as malignancy, opportunistic infections and metabolic toxicity. Thus, novel clinically applicable strategies are needed to induce CTA tolerance. This study assesses the effect of rapamycin (Rapa)-conditioned GM-CSF dendritic cells (DC) in induction of long-term survival in a rat orthotopic hind limb allotransplant model.

**Methods:** Lewis rat bone marrow was cultured for eight days in GM-CSF with or without rapamycin to generate DC. Orthotopic hind-limb transplantations were performed (day 0) from Wistar-Furth to Lewis rats (n=6/group). Controls included group 1: untreated, group 2 cyclosporine (CsA 10mg/kg day 0-20), group 3: Anti-lymphocyte (ALS day -4, +1 and CsA). Experimental group 4: CsA +ALS with rapamycin conditioned GM-CSF DC (Rapa DC) un-pulsed and group 5: CsA + ALS with Rapa DC pulsed with donor antigen (Ag). DC (5 x 10^6) were given on days +7 and +14. Progressive epidermolysis/desquamation of donor skin was considered as end point. Donor alloreactivity was measured using mixed lymphocytic reaction. Intracellular staining for IL-10 and IFN-gamma was performed to assess recipient T cell cytokine production.

**Results:** Rapa conditioned DC were immature. Pulsing the DC with donor antigen did not change the phenotype or function. Donor antigen pulsed Rapa DC significantly prolonged mean hind limb allograft survival to 95.7 days compared to GM-Rapa-DC (45.1 days) and controls (9.3 days). T cells from prolonged rats were hypo responsive to donor Ag and reactive to third party Ag and produced more IL-10.

**Conclusion:** Donor antigen pulsed rapamycin conditioned host DC combined with short-term immunosuppression significantly prolong composite tissue allograft survival across a full MHC mismatch. Thus, reduction of chronic immunosuppression would facilitate the use of CTA as a routine surgical reconstructive option.

15A

TOPICAL TACROLIMUS THERAPY ELIMINATES THE NEED FOR LONG-TERM SYSTEMIC IMMUNOSUPPRESSION IN COMPOSITE TISSUE ALLOTRANSPLANTATION

**Presenter:** Mario G. Solari, MD  
**Authors:** Solari MG, Sacks JM, McLean KM, Hautz T, Horibe E, Unadkat J, Feili-Hariri M, Lee WP  
**University of Pittsburgh**

**Introduction:** Skin is the most immunogenic component of a composite tissue allograft (CTA). Clinically this has manifested as multiple acute skin rejection episodes in most of the human CTA performed to date. Intravenous steroid pulses and increased systemic immunosuppression have been used to mitigate these rejection episodes. Topical immunotherapy is an attractive and practical therapeutic option to provide local immunosuppression with minimal systemic toxicity. The present study was performed to investigate the potential of topical tacrolimus to maintain a CTA after total withdrawal of systemic therapy.

**Methods:** Wistar Furth to Lewis (full MHC mismatch) orthotopic hind limb transplants were performed. Groups included: I- topical tacrolimus alone, II- anti-lymphocyte serum (ALS, 0.5mL) (2 doses) + 21 days cyclosporine (CsA 10/mg/kg/day), III- ALS (2 doses) + 21 days CsA + topical tacrolimus once daily. Biopsies of skin, muscle, and bone were taken for immunohistochemistry and H&E. Co-cultures were performed on days 50 and 100 to assess alloreactivity.

**Results:** All animals in Group I (n=7) developed grade 3 clinical rejection by POD 9, similar to historical controls without treatment. The mean onset of grade 3 rejection was POD 40 with a range of 34-44 in Group II (n=7). In Group III (n=5), two animals developed grade 3 rejection on POD 35 and 56. Three experimental animals are ongoing and rejection free at > 60 days.

**Conclusion:** This study demonstrates the feasibility of maintaining a CTA on topical tacrolimus therapy alone after induction therapy. The induction protocol in this model mirrors what is currently performed clinically at our institution where recipients undergo lymphoid depletion before organ transplantation, followed by weaned immunosuppressive monotherapy. Preoperative depletion of T cells with ALS, along with a short course of systemic immunosuppression, prevents acute rejection, while topical tacrolimus still permits interaction between donor and recipient leukocytes. This novel regimen could reduce or eliminate the morbidity associated with systemic immunosuppression in clinical CTA.
16A
FUNCTIONAL OUTCOME IN FACIAL ALLOTRANSPLANTATION
Presenter: Kia M. McLean, MD
Authors: McLean KM, Solari MG, Sacks JM, Horibe EK, Unadkat JV, Carvell GE, Simons DJ, Lee WP
University of Pittsburgh

Introduction: Face transplantation must provide optimal functional recovery in order to be widely accepted as a reconstructive option. The purpose of this study was to establish an animal model for examining functional recovery in facial allotransplantation. This model is ideal for studying motor regeneration and is novel in its ability to quantify sensory regeneration and cortical re-integration.

Method: Twenty rat hemi-facial flap anatomic dissections were performed to include the functional unit of the rat face. Subsequently, five syngeneic and five allogeneic transplants of the hemi-facial graft were performed. Microvascular anastomoses of common carotid arteries and external jugular veins were performed. Appositions of the infraorbital nerve, buccal and marginal mandibular branches of the facial nerve were performed between donor and recipient. Allogeneic rats were administered 4 mg/Kg/day of intraperitoneal FK-506 for immunosuppression. Nerve conduction studies (NCS) were performed at 5 weeks post-transplant to evaluate motor recovery. Sensory regeneration was studied at 12 weeks post-transplant through microelectrode stimulation of the trigeminal nucleus and quantification with the Whisk 3 computer program. Rats were sacrificed at 20 weeks to examine the re-integration of sensory nerves into the somatosensory cortex, using horseradish peroxidase staining.

Results: Average ischemia time was 85 minutes for all transplants. All animals were drinking and eating on post-operative day one. No animals displayed signs of rejection. All animals showed movement of whiskers on the transplanted side at 2 weeks post-transplant and presence of conduction potentials on NCS at 5 weeks post-transplant.

Conclusion: Motor nerve recovery was confirmed clinically and physiologically. The sensory pathway in each whisker can be traced through the trigeminal nucleus to the somatosensory cortex, quantified with specialized software, and visualized by immunohistochemistry. Using this model, fundamental questions about functional outcome in face transplantation can be investigated.

17A
ENGRAFTMENT OF IMMATURE CD90+ DONOR BONE MARROW CELLS MAINTAINS CHIMERISM DESPITE OSTEOPONTIN DEPENDENT BONE FIBROSIS
Presenter: Maria Siemionow, MD
Authors: Klimczak A, Unal S, Agaoglu G, Siemionow M
The Cleveland Clinic

Purpose: Hand transplants hold great potential in a clinical practice of reconstructive surgery. The bone component of hand may contribute to tolerance induction. We assessed migration of immature (CD90) bone marrow cells (BMC) and role of osteopontin (OPN) following vascularized bone marrow transplantation (VBMT) across MHC barrier.

Method: Thirty-six transplantations were performed between BN(RT1n) donors and Lewis(RT1l) recipients. Group-1 (n=12): isograft between Lewis rats; Group-2 (n=8): allograft without treatment; and Group-3 (n=16): allografts under 7-day alphabeta-TCR/CsA protocol. Flow cytometry assessed immunodepletion, and chimerism for MHC class-I (RT1n) antigens. Immunostaining determined OPN expression in the grafted and recipient bone and donor-derived cells within lymphoid organs. Histology assessed bone architecture.

Results: Isografts showed normal grafted and recipient bones during the entire follow-up (100 days). Group-2 showed BMC reduction in allografted bone (p=0.049). Group-3 displayed viable cells in allografted bone at 21 days post-transplant, however, the viability of BMC declined at day 63 post-transplant (p=0.03). Early engraftment of donor BMC (15.6%) [BN(RT1n)] into the recipient BM compartment was achieved at day 7 post-transplant. Active donor bone hematopoiesis correlated with high chimerism in peripheral blood. Two-way trafficking between donor and recipient BM compartments was confirmed by presence of recipient cells [Lewis(RT1l)] within allografted bone, with peak level of CD90/RT1l at day 21 post-transplant (16.2%–25.7%). At day 63 upregulation of OPN correlated with allografted bone fibrosis. Donor immature cells (3.5%-4.0% of CD90/RT1n) were present in recipient bone and correlated with chimerism maintenance. Donor-derived cells were detected within recipients’ lymphoid organs.

Conclusion: VBMT resulted in efficient engraftment of donor cells into BM and lymphoid organs of recipients. Despite upregulation of OPN expression leading to fibrosis of allografted bone, hematopoietic repopulation of donor cells was taken over by the recipient BM compartment.
18A
A STUDY OF SEQUENTIAL LIMB ISCHEMIA DEMONSTRATES REMOTE POSTCONDITIONING PROTECTION OF MURINE SKELETAL MUSCLE
Presenter: Kyle R. Eberlin, BA
Massachusetts General Hospital

Introduction: Ischemic post-conditioning, the process of exposing tissues to additional brief cycles of ischemia-reperfusion (I/R) after critical ischemia, can mitigate local I/R injury in skeletal muscle (SM). Remote protection of SM has never been demonstrated in post-conditioning I/R models. By varying the duration of ipsilateral reperfusion prior to the onset of contralateral limb ischemia we were able to demonstrate remote post-conditioning protection of murine SM.

Methods: Mice were subjected to 2 hours ipsilateral hindlimb (HL) ischemia followed by reperfusion. Contralateral HL ischemia was subsequently induced for 2 hours after either 0, 20 or 120 minutes of ipsilateral limb reperfusion. All HL ischemia was followed by 24 or 48 hours reperfusion. These groups were compared to animals subjected to simultaneous HL I/R. The gastrocnemius muscles were harvested for histologic evaluation and injury was recorded as injured fibers/total fibers %. Groups were analyzed using an unpaired student’s t-test.

Results: Ipsilateral HLs of animals in the 20-minute interval group had a 59.3% reduction in injury compared to contralateral limbs (16.05±2.41, n=7 vs. 39.47 ± 2.45, n=7) after 24 hours and 61.7% reduction after 48 hours of reperfusion (24.35±2.95, n=7 vs. 63.57 ± 5.49, n=7). In animals with no interval or a 120-minute interval between the onsets of limb ischemia, there was no significant difference in muscle injury between HLs after 24 hours of reperfusion. The injury in these groups was severe and similar to HLs subjected to simultaneous bilateral ischemia and 24 hours of reperfusion (44.83 ± 5.32, n=7).

Conclusion: Sequential ischemia of bilateral HLs leads to similar injury in both HLs if onset of contralateral ischemia is simultaneous, without any reperfusion interval or greater than 120 minutes after ischemia. A 20-minute reperfusion interval between HL ischemia significantly protected against injury in the initial HL subjected to ischemia. This study is the first demonstration of remote post-conditioning of SM and may lead to the development of post hoc therapies.

19A
ISCHEMIC PRECONDITIONING OF MURINE SKELETAL MUSCLE MITIGATES REMOTE INJURY AND MORTALITY
Presenter: Michael McCormack, BS
Massachusetts General Hospital

Introduction: Ischemic pre-conditioning (IPC) has been shown to mitigate I/R injury in a variety of animal models. However, the clinical significance of this protection is less clear and a reduction in mortality has not been previously reported in non-cardiac models. This study was designed to investigate the local and remote protection afforded by IPC skeletal muscle (SM) and to determine the significance of this protection on mortality.

Methods: Mice subjected to 2 hrs hindlimb ischemia and 24 hours reperfusion were compared to mice subjected to a regimen of two 20-minute cycles of IPC followed by standard I/R injury. Mortality was compared in parallel groups followed for 1 week. Local injury was assessed via histology of the gastrocnemius muscles, and injury was recorded as injured fibers/total fibers %. Remote injury was evaluated via histology of intestinal mucosal damage and pulmonary neutrophil infiltration. Groups were analyzed using an unpaired student’s t-test. Mortality differences were interpreted through a Kaplan-Meier survival curve and a hazard ratio.

Results: Significant protection from local and remote injury was observed in IPC animals. There was a 35% reduction in local SM injury compared to WT (71.2% ± 3.4, n=7 vs. 46.0% ± 3.2, n=7, p<0.01), a 50% reduction in remote intestinal injury was observed compared to WT (2.3 ± 0.5, n=7 vs. 1.1 ± 0.1, n=7, p<0.01), and a 43% reduction in remote pulmonary injury was found relative to WT (14.9 ± 1.2, n=7 vs. 8.5 ± 0.7, n=7, p<0.01). IPC animals were also significantly protected from mortality demonstrating a 66.7% survival rate at 1 week compared to 0% survival after standard injury alone (hazard ratio 0.20, 95% CI 0.02-0.59, n=12).

Conclusion: We have developed a murine model of IPC that demonstrates local and remote protection against I/R injury, as well as a reduction in mortality. This study exhibits the first significant reduction in mortality through IPC in a non-cardiac model of injury. This model demonstrates the powerful effect of IPC of SM on local and remote tissues and will facilitate further study of potential mechanisms and therapies.
TOPICAL FK506: CAN IT SURPASS THE ONE OF THE HIGHEST HURDLES IN COMPOSITE TISSUE ALLOTRANSPLANTATION

Presenter: Justin M. Sacks, MD
Authors: Horibe EK, Unadkat JV, Sacks JM, Feili-Hariri M, Lee WP
University of Pittsburgh

Introduction: Topical therapies in composite tissue allotransplantation (CTA) are sought as a potential way to decrease use of systemic immunosuppression while maintaining allograft acceptance. It has been shown that topical FK506 (TFK)0.05%, prolongs non-vascularized skin allograft survival in rats by 14 days. Vascularized skin allografts, however, differ in that they closely reflect the immunological reactions that take place in CTA and despite this, impact of TFK on their acceptance remains unclear. Therefore we studied the effect of TFK on survival of vascularized skin allografts, across a full MHC mismatch in rats.

Methods: Recipients in 4 groups underwent allotransplantation (drugs administered from day 0-20): I) untreated; II) Cyclosporine (10mg/Kg intraperitoneally); III) TFK 0.03% (0.3mg/day); IV) TFK 0.1% (1.2mg/day). Systemic levels of FK506 were measured on POD 6 and 30. Allograft appearance and histology were assessed postoperatively.

Results: Vascularized skin allografts in group VI survived longer (median: 9 days) than in group I (7, p= 0.02) and in group III (6.5, p=0.01). In group IV, 1/6 allograft was accepted until POD34, when it presented with an episode of acute rejection at a biopsy site; the allograft was rescued after topical therapy was resumed, however new episodes continued to occur sporadically; 1/6 allograft was accepted over 100 days. Both animals rejected third-party-derived skin grafts faster than donor-derived ones. Nonetheless, graft survival in group IV was not statistically significant when compared to group II (33, p=0.70). Systemic levels of FK in all cases were below therapeutic range.

Conclusion: TFK0.1% is more effective than TFK0.03% in delaying allograft rejection, however TFK alone is not superior than cyclosporine. TFK0.1% at 1.2mg/day showed minimal systemic absorption and did not cause side effects. Since in vascularized allografts alloantigens are distributed systemically in secondary lymphoid organs and TFK0.1% probably acts locally, association with therapies that target the indirect pathway may be essential to prolong acceptance of vascularized skin allografts.
Friday, June 22, 2007

Session 4A
Fetal Surgery/Wound Healing
Abstracts 21A – 29A
9:00 – 10:20 am

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21A
BFGF GENE TRANSFER BY ADENO-ASSOCIATED VIRAL 2 VECTORS DECREASES WORK OF ACTIVE DIGITAL FLEXION AND ADHESION FORMATION: AN IN VIVO STUDY UP TO END TENDON HEALING STAGE
Presenter: Paul Y. Liu, MD
Authors: Liu PY, Cao Y, Zhu B, Tang JB, Xin KQ, Wang XT
Roger Williams Medical Center

Purpose: Previously, we demonstrated that adeno-associated virus-2 (AAV2) mediated gene transfer promotes expression of collagen genes and enhanced healing strength at 4 weeks post-surgery. In this study, we investigated effects of delivery of the bFGF gene to injured flexor tendon in a clinically relevant model on several ultimate outcome measures at end tendon healing stage -- ultimate gliding function, work of digital flexion and extent of matured adhesions.

Methods: We used 20 long toes from 10 white leghorn chickens. These toes were randomly divided into 2 groups of 10 each. The flexor digitorum profundus (FDP) tendons were cut completely in zone 2 and were repaired with modified Kessler method. In AAV2-bFGF group, a total of 2 X10^9 particles of AAV2 harboring the bFGF gene were injected to both stumps of the tendons. In non-treatment group, the tendons were repaired, but no injection was given. The toes were immobilized for 3 weeks and were released to allow free motion thereafter. At 12 weeks, the energy required to flex the toe (work of flexion) was tested in a tensile testing machine and gliding excursion of the FDP tendon was measured. Adhesions were recorded according to scoring criteria.

Results: The work of flexion of the toes in the AAV2-bFGF treatment group (0.021 +/- 0.006 J) was significantly less than that of non-treatment controls (0.033 +/- 0.015 J) (p < 0.05). The gliding excursion of the AAV2-bFGF treated FDP tendons was not significantly changed compared with that of the tendons in non-treatment group. Adhesion scores of the AAV2-bFGF group (2.8 +/- 0.7 points) were significantly less than those of the control tendons (3.8 +/- 0.9 points)(p < 0.05).

Conclusions: bFGF gene transfer via AAV2 vectors to digital flexor tendon significantly decreases energy required to flex the digits and adhesion formations. We evaluated the outcomes at the end stage when adhesions and healing had matured and function was steady. The findings suggest that delivery of bFGF gene through AAV2 has advantage of decreasing adhesion formation during tendon healing process and benefits ultimate digital motion.

22A
HUMAN BETA-DEFENSIN-3 GENE TRANSFER TO WOUND INFECTIONS IN DIABETIC PIGS
Presenter: Malte Spielmann, MS
Authors: Spielmann M, Hirsch T, Zuhaili B, Fossum M, Steinau HU, Yao F, Onderdonk AB, Steinstraesser L, Eriksson E
Brigham & Women’s Hospital of Harvard Medical School

Introduction: Wound infections in diabetic patients are becoming more common. Increasing morbidity and high numbers of poly-resistant microbes underline the need for the development of new therapeutic options. Host Defense Peptides are known as part of the human innate immune system. Particularly human Beta Defensin-3 (hBD-3) has broad antimicrobial and immunomodulatory activity. We hypothesize that transient overexpression of hBD-3 in infected diabetic wounds lower bacterial burden in wound tissue.

Methods: 14 incisional wounds were created on the back of each diabetic Yorkshire pig. Wounds were microseeded using adenoviral vectors coding either for hBD-3 transgene or LacZ reporter gene as control. The wounds were enclosed in a sealed polyurethane chamber and wounds were inoculated with 2x10^8 CFU Staphylococcus aureus. Tissue biopsies were taken for histological and microbiologic analysis.

Results: Our large animal model showed a stable S. aureus infection over the time course of the experiment (>10^5 CFU/g tissue; 12 days). The hBD-3 gene transfer study group showed significant lower (p=0.005) bacterial burden (2.9x10^6) in the wound tissue after 4 days compared to control wounds (2.3x10^7). Reduction of bacterial counts in the tissue remained for the duration of experiment and showed 5.27 x10^5 CFU /g tissue for hBD-3 study group, whereas control wounds showed 6.35x10^6 CFU/g tissue.

Conclusion: This study demonstrates that transient overexpression of human Beta-Defensin-3 in infected diabetic wounds is able to reduce bacterial counts in the wound tissue for 12 days by 10 fold. However, we could not eliminate the infection completely by the therapeutic approach employed. Further studies are necessary to optimize the therapeutic approach of application of human Beta-Defensin-3 and to gain a better understanding of its immunologic function in infected wounds.
23A  STEM CELL THERAPY ACCELERATES DIABETIC WOUND HEALING

Presenter:  Clarence D. Lin, MA
New York University School of Medicine

Introduction: Bone marrow (BM)-derived stem cells play an important role in ischemic tissue repair and wound healing. In diabetics, BM progenitor cell function is known to be impaired. We hypothesize that wild-type (wt) ex vivo expanded lineage negative (Lin-) BM cells improve diabetic (db) wound healing.

Methods: Uncommitted wt Lin(-) BM progenitor cells were magnetically separated. These cells were tagged with Dil, mixed in a collagen gel and applied to an established excisional db wound healing model. Wounds treated with lineage positive cells served as a control. Wounds were grossly and histologically analyzed for time to closure, epithelial gap, granulation tissue formation, cell proliferation, and vascularity. Immunohistochemistry was performed with anti-CD31 antibody.

Results: Db wounds treated with wt Lin(-) ex vivo expanded BM cells showed a statistically significant decrease in time to closure relative to Lin(+) treated controls (100% vs. 73% at 21 days; p<0.05). Moreover, wt Lin(-) treated wounds had statistically smaller wound surface area at all time points (p<0.05). Histologically Lin(-) treated wounds had a statistically smaller epithelial gap at harvested time points (p<0.05). Proliferating Dil-positive wt donor cells were present and viable in db recipient tissue at 21 days; furthermore, co-staining with CD31 demonstrated that wt Lin(-) cells differentiated into endothelial cells and formed new blood vessels. These wt Lin(-) cells also contributed to a statistically higher vessel count per high power field compared to control wounds.

Conclusions: Topical delivery of wt Lin(-) BM cells results in a statistically significant reduction in time to diabetic wound healing. Donor cells proliferate and remain viable in the db recipient wound. Delivery of BM-lineage negative cells may provide a therapeutic modality to improve wound healing in diabetics.

24A  AGE-RELATED CHANGES IN THE MOBILIZATION OF ENDOTHELIAL PRECURSOR CELLS IN HUMANS-EFFECT OF DISEASE COMPLICATIONS

Presenter:  Edwin Chang, PhD
Authors:  Chang E, Paterno J, Sabor S, Chang E, Kelantan M, Cooke J, Birke K, Gurtner G
Stanford University

Introduction: We showed previously that undiseased humans have constant circulating human endothelial precursor cells (EPC) levels with respect to age. The literature describes declines in circulating EPCs numbers with respect to age, however, the latter findings stem from studies of individuals possessing either cardiovascular and/or diabetic complications. We hypothesize that such complications may mimic a systemic wound healing phenotype that continually induces the mobilization of EPCs into the circulation and that human aging is characterized by an attenuated mobilization potential for circulating EPCs.

Methods: Human blood was obtained from patients before and after a 30 minute treadmill session. Mononuclear cells were isolated by Ficoll-Paque density gradient centrifugation. EPC prevalence was determined via flow cytometric analysis. CFU-E colony forming potential was measured through commercially available Hill assay kits. Circulating mononuclear cells and putative EPCs were also measured for the parameters of: telomerase activity, telomere length, proliferation, migration and HIF-1α.

Results: EPCs appeared at levels of 0.1 % in the circulation and such cells could be induced with 30 minutes of exercise. For young donors (21-44 yrs), the prevalence of these types of cells increased 6-fold after exercise but such mobilization became attenuated with respect to age. Basal prevalence remained unchanged. There was less classical Hill colonies after exercise but analysis of the isolated colonies showed an increase in the occurrence of the AC133-based EPC phenotype. Telomerase was also present in the colonies.

Conclusions: We found a systemic loss in EPC mobilization as a result of aging consistent with a diminished response to a stress stimulus. Baseline mobilization of EPC remains unchanged. These two observations serve to resolve the apparent contradictions between our previous studies and those reported in the literature. There is some evidence that aging affects EPC behavior directly although our studies in the plasma suggest a more systemic age-related alteration.
25A
DIABETIC WOUND HEALING RESPONSE TO CD-34-SORTED AND UNSORTED ADIPOSE-DERIVED STROMAL CELLS DELIVERED AS SELF ORGANIZING MESENCHOID BODIES

Presenter: Peter J. Amos, BS
University of Virginia

Introduction: Adipose-derived stromal cells (ASCs) appear to confer regenerative benefits when introduced into various injured tissues, including bone, cartilage, and myocardium. We hypothesize that ASCs possess the ability to enhance healing of diabetic wounds. We have developed a novel method for culturing ASCs as self-organizing mesenchoid bodies (SOMBs), and here we test their ability to expedite healing of full thickness wounds in diabetic mice, focusing on CD-34 as a putative marker of wound healing efficacy.

Methods: Human ASCs were isolated from an elective lipoectomy specimen using well-documented methods. Cells were cultured and sorted (P=3) for CD-34 expression. CD-34-positive, CD-34-negative, and unsorted ASCs were grown as SOMBs (25,000 ASCs/SOMB) for 8 days in serum-free medium. On Day 0, a 1 cm diameter full thickness excisional cutaneous wound was made on the back of homozygous diabetic null mice. Each wound was treated in a blinded fashion on post-wounding day 1 with 5 SOMBs delivered topically in ~20 ml PBS under a Tegaderm dressing in the following groups: CD-34-positive SOMBs (N=7), CD-34-negative SOMBs (N=9), and unsorted SOMBs (N=4). Digital images were taken periodically until Day 21, and open wound area, expressed as a percentage of initial wound area, was quantified.

Results: Wound areas in all groups were statistically similar at each timepoint, suggesting that prospective sorting based on CD-34 expression had no impact on wound healing. Healing rates in ASC SOMB-treated wounds were statistically similar to those in diabetic mice treated with vehicle control for the first week after wounding, but by day 9, all diabetic wounds treated with ASC SOMBs were significantly smaller than those in diabetic mice receiving vehicle control and statistically similar to wounds in wild type non-diabetic mice.

Conclusion: Administration of ASCs as SOMBs accelerates wound healing in diabetic mice. Prospective ASC enrichment on the basis of CD-34 expression did not enhance this therapeutic effect. Our findings suggest promise for ASCs as a novel therapeutic approach to healing difficult wounds.

26A
MICROARRAY ANALYSIS OF DIABETIC RAT WOUNDS TREATED WITH EITHER V.A.C.® THERAPY, BLUESKY, UNDER SUCTION OR MOIST WOUND HEALING

Presenter: Kathleen Derrick, MS
Authors: Derrick K, McNulty A, Norbury K, Kieswetter K
Kinetic Concepts, Inc.

Introduction: Vacuum Assisted Closure® Therapy (V.A.C.® Therapy) is extensively used to treat both acute and chronic wounds. Use of this negative pressure therapy is widely known to create an environment that promotes wound healing. Whole-genome studies were designed to gain insight into the biological processes which V.A.C.® Therapy may affect and how the processes may differ between V.A.C.® Therapy and an alternative form of negative pressure therapy.

Methods: One 3 cm wound was made dorsally on each of 12 diabetic rats. Wounds were treated per manufacturer guidelines with either V.A.C.® Therapy (-125mm Hg, continuous) using V.A.C.® GranuFoam Dressing, gauze under suction (Versatile™ Wound Vacuum System -75 mm Hg, continuous), or with moist wound healing (Tegaderm™; 3M™). After 2 days of continuous treatment, wound tissue was harvested. The Applied Biosystems microarray platform was used to assess gene expression for all 24 pieces of tissue (12 baseline tissues from wound creation, 12 wound tissues from day 2 post treatment).

Results: After differential analyses, data were entered into the PANTHER™ classification system (www.pantherdb.org) to assess biological processes which were differentially affected by the three therapies. V.A.C.® Therapy showed a significant difference in structural proteins (p=1.91x10^-10) where no change was seen in the other two treatment groups. Significantly more glycolysis related genes were expressed than expected following V.A.C.® Therapy (p=8.58x10^-6) compared to gauze under suction (p=0.003). Other significant signaling pathways affected following V.A.C.® Therapy were TGF-beta signaling (p=0.02), cadherin (p=0.03), FGF (p=0.008), purine metabolism (p=0.0001), and FAS (TNF superfamily; p=0.006).

Conclusion: These data show that V.A.C.® Therapy has a major influence on biological processes expressed during wound healing in a manner which significantly differs from both gauze under suction and moist wound healing.
PULSED ELECTROMAGNETIC FIELDS ENHANCE WOUND HEALING BY AUGMENTING ENDOTHELIAL PROGENITOR CELL FUNCTION AND FGF-2 EXPRESSION

Presenter: Eric I. Chang, MD  
Authors: Chang EI, Aarabi S, Gurtner GC  
Stanford University School of Medicine

Introduction: Pulsed electromagnetic fields (PEMF) have been shown to promote wound and fracture healing. We have recently demonstrated that electrical fields promote new blood vessel growth and diabetic wound healing. Since endothelial progenitor cells (EPCs) are known to contribute to new blood vessel growth, the purpose of this study was to determine the effects of electromagnetic fields on progenitor cell function.

Methods: Full thickness, 5mm-diameter wounds were created on the dorsum of athymic nude mice injected with diI-labeled, human EPCs which were then exposed to either PEMF (4.5 ms pulse/15 Hz) for 12 hours/day or to normal environmental conditions. Wound closure was measured at various timepoints. Immunohistochemical examination assessed CD31+ vascular density and incorporation of EPCs. The mobilization of Flk1+/CD11b- endothelial progenitor cells (EPC) was examined at day 3. Human EPCs were exposed to PEMF in vitro to determine whether there was any proliferative effect or upregulation of FGF-2 expression.

Results: EPCs in vitro showed a significant increase in proliferation and a three-fold increase in FGF-2 expression when exposed to PEMF. Similarly in vivo wound healing was significantly increased in those mice exposed to electrical fields compared to controls (7 days vs. 12 days, p<05) with a substantial increase in CD31 vessel density (vessels/HPF, 30±7 vs. 52±13, p<.05). The exposed mice demonstrated a higher percentage of EPC mobilization compared to normal controls (6.91±.55 vs. 4.38±.79, p<.05).

Conclusions: Pulsed electromagnetic fields increased EPC proliferation and FGF-2 expression in vitro and increased vascular density in vivo to augment wound healing. This increased vascular density is likely a result of both increased angiogenesis and vasculogenesis. Thus PEMF promote neovascularization in part by enhancing EPC function.

EXPRESSION OF THE A-SMA GENE AND CONTRACTILE ABILITY OF SKIN FIBROBLASTS FROM DIABETIC MICE: CORRELATION WITH WOUND CLOSURE RATES IN VIVO AND IMPLICATIONS IN WOUND HEALING

Presenter: Xiao Tian Wang, MD  
Authors: Wang XT, Rieger-Christ KM, Summerhayes IC, Gao JS, Tang J, Liu PY  
Roger Williams Medical Center

Purpose: Healing potential of the wounds in diabetic patients is very limited and delay or non-healing of diabetic wounds are serious problems that lack efficient treatments clinically. We investigated changes of a-SMA gene expression in diabetic skin fibroblasts and contractile ability of collagen gel matrix seeded with the fibroblasts and correlations with in vivo wound closure rate, and propose gene therapy approaches to reverse detrimental efforts.

Methods: We used 10 db+/-db- diabetic mice (BKS. Cg-m+/-1 Leprdb) and 10 littermates. Two skin excision wounds, 0.8 x 0.8 cm each, were created on the back of each mouse, and sizes of the wounds were recorded over post-surgical 4 weeks. Excised skin was cultured to obtain skin fibroblasts. The fibroblasts were seeded into three-dimensional collagen gels and gel dimensions were recorded for a 3-week period. RNA of skin fibroblasts was extracted to assess the levels of expression of the a-SMA gene. We transferred to diabetic skin fibroblasts VEGF or PDGF genes and determined the effects of gene therapy.

Results: The closure rate of the skin excision wounds was significantly greater in the db+/db+ mice (20.4 +/- 2.1 days) than in their littermates (15.5 +/- 1.9 days) (p < 0.001). The fibroblasts from the db+/db+ mice showed very poor contractile behavior in the three-dimensional collagen gels; in contrast, the gel seeded with the non-diabetic fibroblasts shrinked remarkably. The differences in dimensions of gels are significant at 1, 2, and 3 weeks (p < 0.001). Levels of a-SMA gene expression were significantly lower in the db+/db+ diabetic skin fibroblasts than in the cells of their littermates (p < 0.05) and a-SMA gene expression was significantly elevated by transfer of the VEGF and PDGF genes.

Conclusions: Down-regulation of a-SMA gene expression may be responsible for the lower contractile ability of diabetic skin fibroblasts and delay in closure of the skin wounds. This study demonstrated that transfer of growth factor genes through appropriate gene therapy approaches increases a-SMA gene expression and can be a potential method to enhance healing of diabetic wound.
29A
THE ROLE OF THE DURA MATER IN BMP2-MEDIATED RECONSTRUCTION OF LARGE-SCALE CALVARIAL DEFECTS
Presenter: Darren M. Smith, MD
Authors: Smith DM, Afifi AM, Cooper GM, Mooney MP, Losee JE
University of Pittsburgh

Introduction: Pediatric craniofacial surgery is complicated by a shortage of autologous bone for the repair of congenital or trauma-induced osseous defects. Recombinant human Bone Morphogenetic Protein-2 (rhBMP2) on collagen scaffolds has demonstrated osteoinductive potential in spinal fusions and is in the early stages of acceptance by craniofacial surgeons. This study demonstrates the efficacy of rhBMP2 in large-scale calvarial defect repair and addresses the role that dura mater plays in facilitating this process.

Methods: Square defects (15 x 15 mm) were made in the calvaria of 13 adult New Zealand White rabbits. The rabbits were divided into three groups: untreated controls (n=6); rabbits treated with rhBMP2 on a collagen sponge (INFUSE, Sofamor Danek) (n=5); and rabbits treated with rhBMP2 on a collagen sponge after a 15mm square patch of dura was excised beneath the calvarial defect (n=2). Bone regeneration was evaluated 6 weeks postoperatively by 2D and 3D CT scan and quantified using ImageJ. Statistical analysis (ANOVA) was performed in SPSS.

Results: The rhBMP2-treated animals with intact dura healed an average of 96.6% of the defect area by six weeks, while the rhBMP2-treated animals without dura underlying the defect healed an average of 80.6%, and the empty controls healed an average of 32.8%. This represents a statistically significant difference in healing potential between all groups by ANOVA (F=129.6, p<0.001). Qualitative analysis of coronal CT scans revealed the bone generated in animals with dura to be appreciably more robust than that generated in animals with dural excisions.

Conclusions: This study demonstrates the effects of rhBMP2 in collagen sponges on the repair of calvarial defects in the rabbit model. Consistent with its known reservoir of osteoblastic precursors, the dura mater appears to play an important role in contributing to rhBMP2-mediated calvarial defect repair. These results suggest that rhBMP2 may offer a viable treatment option for craniofacial surgeons facing a shortage of autologous bone graft donor tissue.
Friday, June 22, 2007

Session 5A
Craniofacial/Bone
Abstracts 30A – 35A
11:00 – 11:50 am

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30A
TENSILE STRAIN OF SUTURE CELLS CAUSES ETS-2 PHOSPHORYLATION BY CAMKII AT THREE SITES: SERINE 246, SERINE 310, AND SERINE 313
Presenter: Jack C. Yu, MD
Authors: Yu JC, Chen JR, Winger J, Fulzele S, Wenger KH, Borke JL
Medical College of Georgia

Introduction: Mechanotransduction is the conversion of mechanical forces into biochemical signals. It underpins the optimization and homeostasis of the craniofacial skeleton. Intracellular calcium concentration and peptide phosphorylation status are two key proximate parameters used in mechanotransduction. In this study, we attempt to construct the early intracellular events resulting from cellular strains. The objectives are to establish a chain of causality linking cellular strain to substrate phosphorylation and to identify both the agent and the target of phosphorylation.

Methods: Cyclic tensile force (0.5N at 1 Hz) was applied to 1 day old rat sagittal sutures. Intracellular calcium concentration showed immediate increase as measured by FURA-2. Cytosolic calcium increase results in CaMKII (Ca-dependent Calmodulin Kinases II) binding with its substrate, Ets-2. Ets-2 is a transcription factor for many genes important in osteogenesis. We showed CaMKII causing Ets-2 phosphorylation by western blot autoradiography. Peptide array (nanopeptides, n=22) was then constructed to determine the precise sites of phosphorylation. The results were confirmed with MALDI-TOF (Matrix-Assisted Laser Desorption/Ionisation-Time of Flight) Mass Spectroscopy.

Results: There are three consensus sites for phosphorylation of Ets-2 by CaMKII: RVPS, FESF, and RLSS; all three contain serine residue. Serine 246, 310, and 313 were the targets. MALDI-TOF Mass Spectroscopy of the selected nanopeptides treated by CaMKII showed 80 Da right shifts consistent with phosphorylation. There is significant nuclear translocation of Ets-2 upon tensile deformation of the suture cells. CaMKII binding of Ets-2 occurred within 30 minutes after the onset of tensile strain.

Conclusion: One of the means for cranial suture cells to respond to tensile forces is by increase in intracellular calcium which causes CaMKII to phosphorylate Ets-2 resulting in Ets-2 nuclear translocation. Of note, Ets-2 is at the intersection of three key signaling pathways important in the pathogenesis of craniosynostosis: FGF-2, TGF-beta, and mechanotransduction.

31A
EX VIVO NOGGIN GENE THERAPY INHIBITS POSTOPERATIVE RESYNOSTOSIS
Presenter: Gregory M. Cooper, PhD
Authors: Cooper GM, Usas A, Olshanski A, Mooney MP, Losee JE, Huard J
University of Pittsburgh

Introduction: Resynostosis following surgical correction of primary craniosynostosis necessitates further surgical intervention, increasing morbidity and mortality. Bone morphogenetic proteins (BMPs) are known to be expressed during normal bone healing. This study tested the hypothesis that treatment of sutectomy sites with Noggin, an extracellular antagonist of BMPs, would inhibit postoperative resynostosis in a mouse sutectomy model.

Methods: One hundred-eight, 10 week old C57BL/6J mice (Jackson) were used in this study. Sutectomies (1mm x 0.5mm) were performed on the fused posterior interfrontal sutures and animals were assigned to one of three groups. Sutectomy sites were left untreated in Group 1 (n=36). Group 2 sites were treated with 4x10⁵ cells expressing GFP (n=36). Group 3 sites were treated with 4x10⁵ cells expressing both Noggin and GFP (n=36). Defect healing was assessed radiographically (MX-20, Faxitron X-Ray Corp.) or by micro-computed tomography (µCT; VivaCT40, Scanco, USA) 0, 4, 8, and 12 weeks postoperatively. Defect areas (from radiographs) and defect volumes (from µCT) were measured and means and standard deviations were compared among groups and postoperative time points. Significant differences were determined using SPSS software.

Results: Radiographic analysis showed that Noggin treated sutectomy sites were significantly larger than untreated sites 4 and 8 weeks postoperatively (p<0.05). Analysis of defect volume showed that Noggin treated defects were significantly larger than untreated defects at all time points after surgery. The GFP treated defects demonstrated some inhibition of bone formation, but this inhibition was not significant compared to untreated controls 12 weeks after surgery.

Conclusions: These findings suggest that Noggin is an effective inhibitor of bone formation within small sutectomy sites and that Noggin may be useful in avoiding postoperative resynostosis. Noggin treatment may be useful as an adjunct to traditional surgical intervention for the treatment of children with craniosynostosis.
**32A**

**RADIATION IS A KEY REGULATOR OF ANGIogenic AND ANGIostatic CXC CHEMOKINE EXPRESSION IN HUMAN ENDOTHELIAL CELLS**

**Presenter:** Carrie L, Scharf, BA  
**Authors:** Chang CC, Scharf CL, Thanik VD, Lerman OZ, Greives MR, Macklin J, Hazen A, Warren SM, Levine Saadeh PB  
New York University School of Medicine

**Introduction:** Blood vessel growth is regulated through complex signaling pathways involving angiogenic and angiostatic CXC chemokines. These signals are modulated by various stimuli including hypoxia and inflammation. Increasing evidence suggests that low-dose ionizing radiation (XRT) may be a vasculogenic stimulus. We investigated the effect of XRT on the patterns of CXC chemokine signaling in human endothelial cells.

**Methods:** Vascular endothelial cells (HUVECs) were exposed to 0, 5, or 20Gy XRT. 24-48hrs after XRT, total cell protein lysate was harvested and cytokine expression was analyzed by antibody array. Candidate cytokine upregulation was confirmed by quantitative real time RT-PCR and ELISA. Annexin V and CXC chemokine receptor (CXCR) profiles were analyzed by flow cytometry.

**Results:** All vasculogenic chemokines (CXCL1-3/5-8) demonstrated a 3-13 fold dose-response induction when exposed to 5Gy (low dose) and 20Gy (high dose) XRT, respectively. PDGF and VEGF demonstrated 2 and 3-fold induction when exposed to 5Gy and 20Gy, respectively. Inflammatory cytokines IFN-gamma (1.5-fold) and TNF-beta (3-fold) were only upregulated at 20Gy. Interestingly, 20Gy XRT also led to marked increase in angiostatic CXC chemokine production. Annexin V assay demonstrated a dose-dependent decrease in apoptosis (0Gy = 16% vs. 5Gy = 4.5%), however 20Gy markedly increased Annexin V expression (24%). Dose-dependent CXCR4 expression increased 8 and 16-fold at 24 and 48hrs after XRT. CXCR1-3 expression did not change in response to XRT.

**Conclusion:** XRT induces a dose-dependent increase in angiogenic CXC chemokines and their receptors. Interestingly, inflammatory mediators and angiostatic chemokines were induced only at high dose XRT. This suggests that XRT induces an angiogenic profile at low dose and a proinflammatory, angiostatic and apoptotic profile predominates at high dose. Ongoing experiments are elucidating the mechanism by which CXC chemokine expression balances angiogenic and angiostatic effects in response to XRT in order to identify novel targets for therapeutic intervention.

**33A**

**RADIOPROTECTION OF PRIMARY OSTEOBLASTS BY AMIFOSTINE AND A FRACTIONATED DOSE REGIMEN**

**Presenter:** Alex K. Wong, MD  
**Authors:** Wong AK, Mei L, Soares MA, Li S, Mehrara BJ  
University of Pittsburgh Medical Center

**Introduction:** Fractionation and amifostine (a free radical scavenger) have been used clinically to reduce the morbidity of radiation therapy. However, the cellular mechanisms governing the radioprotective effects of these treatments remains unknown. The purpose of this study was analyze the cellular mechanisms by which these modalities provide radioprotection for primary osteoblasts.

**Methods:** Primary osteoblasts were pre-treated with 4 mM amifostine or vehicle for 30 minutes and then exposed to either a single dose of 7 Gy of radiation or 3 divided doses of 2.33 Gy (a total of 7 Gy over 72 hours). Cell viability was measured using an MTT assay. To measure osteoblast function, we tested the ability of osteoblasts to produce VEGF in response to hypoxia using ELISA. We also assayed for osteodifferentiation by measuring alkaline phosphatase (ALP) activity.

**Results:** Cell survival was improved by 61% and 48% by fractionation and amifostine, respectively (p<0.05). Combination therapy produced 100% radioprotection of viability (P<0.01). While hypoxia induced VEGF was decreased by 63% after a single dose of 7 Gy, fractionation resulted in only a 15% decrease (P<0.05). Interestingly, amifostine had no cytoprotective effect on hypoxia induced VEGF production. A single dose of 7 Gy produced a 3.7-fold decrease in ALP activity relative to unirradiated cells. Importantly, neither amifostine, fractionation or combination therapy resulted in significant preservation of osteoblastic differentiation potential.

**Conclusions:** While both fractionation and amifostine prevent negative effects of radiation on cell viability, combined the radioprotective effects these two modalities are additive. Fractionation but not amifostine significantly preserves the ability of osteoblasts to properly respond to hypoxia by producing VEGF. However, neither therapy prevents radiation induced inhibition of differentiation. This finding is the first demonstration that osteoblast survival and differentiation are distinctly different phenomena and may provide an explanation for the variable clinical effects noted with these treatments.
BIOMECHANICAL ASSESSMENT OF REGENERATE INTEGRITY IN RADIATED MANDIBULAR DISTRACTION OSTEONEOGENESIS (MDO)

Presenter: Ameen M. Jamali, BS
Authors: Jamali AM, Schwarz DA, Kakwan MS, Arman KG, Buchman SR

University of Michigan

Introduction: The use of MDO for tissue replacement after oncologic resection or as a reconstructive option for deformations secondary to irradiated bone could have immense potential therapeutic ramifications. The role of MDO for reconstruction of mandibular defects following therapeutic radiation (XRT) will depend on determining the quality and extent of attenuated bone healing in the regenerate (RG). Although we have previously shown radiographic evidence of bony union after XRT in a rat model of MDO, biomechanical testing remains the gold standard to measure the degree of RG integrity. The purpose of this study was to determine the biomechanical properties of the RG after XRT using yield (Y) and breaking load (BL). We hypothesized that both biomechanical healing variables would be significantly reduced in XRT-MDO compared to MDO.

Methods: Male Sprague-Dawley rats underwent either 10day fractionated 36Gy pre-operative external beam XRT to the left mandible followed by 2 weeks recovery (n=7), or no XRT (n=10) before surgery; External fixators were secured and unilateral osteotomies were created behind the 3rd molar. MDO was performed after 4days latency at 0.3mm Q12hrs followed by 4wks consolidation. Mandibles were potted using Bismuth Alloy then tension tested at 0.5mm/s to failure using the 858 MiniBionix MTS machine. Y and BL were determined; a p-value of < 0.05 was considered significant.

Results: There was a significantly lower BL for XRT-MDO compared to MDO. However we found no significant difference in yield between the groups.

Conclusion: The diminished BL of XRT-MDO implies that the inductive effects of MDO do not overcome the attenuation in strength caused by XRT. This lowered BL in XRT-MDO reflects the reduced biomechanical quality of the regenerate bone, despite evidence of radiographic union. Our data clearly show that simple radiographic union after XRT-MDO is not an adequate outcome measure to evaluate RG healing. This supports the importance of defining quantitative bone healing metrics in prior to attempts at implementation of MDO after XRT in clinical head and neck reconstruction.

THE DIFFERENTIATION OF ACUTE, CHRONIC AND DIABETIC WOUNDS BY MUDPIT ANALYSIS

Presenter: Christopher Sleyman, MS
Authors: Sleyman C, Kyas A, Jacobsen F, Wolters D, Voigt A, Klein HH, Steinau HU Steinstraesser L

BG University Hospital Bergmannsheil, Ruhr University Bochum

Introduction: Although there have been many investigations into the mechanisms and context of wound healing, the process is still not completely understood. Former approaches focused on the elucidation of the role of individual proteins. Our approach instead is to use proteomic techniques to find differences in the proteome of acute wounds, chronic bed soars, and diabetic ulcers and conduct a comparative analysis.

Methods: Wound fluids (acute, diabetic or chronic) are collected, processed to deplete abundant proteins, concentrated and investigated by MudPIT analysis (multidimensional Protein Identification Technology, which utilizes LC/LC-MS/MS ESI-mass spectrometry). The resulting MS/MS-spectra are further processed using the SEQUEST algorithm and database search, yielding lists of candidate proteins. These results are put into a large database which allows easy access to and analysis of the identified proteins under aspects of diverse biological and physicochemical parameters and connections.

Results: With an average of more than 300 proteins identified per run we are already able to provide a more detailed insight into the proteome of acute and chronic wounds. So far, more than 600 different proteins could be identified in different wound fluids showing that the protein profile of acute wound fluid resembles the serum protein profile to a high degree but differs strongly from the profile of diabetic and chronic wounds. The latter resemble each other to a wider extent.

Conclusion: Our results reveal differences in the protein profile of acute and chronic wounds. The extent of individual variation has still to be evaluated, nonetheless, our results imply that on a larger sample range our proteomic approach can depict system inherent differences between the two physiological wound states. These differences allow deeper insight into the mechanism of normal and impaired wound healing. Furthermore it raises the possibilities to identify valuable biomarkers for new strategies of wound therapy.
Friday, June 22, 2007

Session 6A
Tissue Engineering
Abstracts 36A – 50A
1:00 – 3:20 pm

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ENHANCING NEOVASCULARIZATION AND SUBSEQUENT OSTEOGENESIS IN TISSUE-ENGINEERED BONE GRAFTS

Presenter: Leila Jazayeri, BS, BA
Authors: Jazayeri L, Roldan JC, Kelantan M, Chang E, Fong K, McMahon D, Longaker MT, Gurtner GC

Stanford University

Introduction: Bone generation via implantation of bone graft substitutes (BGS) is a promising surgical technique. Poor vascularization of BGS, however, results in inadequate osteogenesis and graft resorption. Human umbilical vein endothelial cells (HUVECs), mesenchymal stem cells (MSCs), and vascular endothelial growth factor (VEGF) enhance neovascularization in soft tissue. This study thus investigates their ability in combination with BGS to enhance neovascularization and subsequently ectopic osteogenesis.

Methods: In vitro. MSCs, HUVECs, or no cells were seeded onto 5 different BGS with BMP-7 for 3 wks. Culture media was taken on days 1, 3, 5, 7, 14, and 21 and analyzed with ELISA for VEGF, stromal derived factor (SDF-1), nitric oxide (NOx), bone alkaline phosphatase (BAP), osteocalcin (OC), and bone morphogenic protein 2 (BMP-2). Seeded BGS were imaged with confocal laser microscopy and scanning electron microscopy (SEM). In vivo. Blocks of BGS were seeded with MSC and BMP (n=10), VEGF and BMP (n=10), or BMP alone (n=10) and subcutaneously implanted in mice. At 5wks BGSs will be explanted and analyzed for neovascularization and osteogenesis, with H&E, immunostaining for CD31 and Von Willebrand, histomorphometry, and 3D confocal imaging.

Results: Confocal and SEM reveal superior MSC (vs HUVEC) migration and adherence to BGSs. MSC seeded BGS secreted an impressive panel of pro-angiogenic markers by d7 including high levels of VEGF and SDF-1 with moderate NOx. Both VEGF and SDF-1 accumulated overtime (p<0.01). Day 7 osteogenic markers, however, were minimal; moderate BAP with no BMP-2 or OC was detected. Animal studies are ongoing.

Conclusions: We show that MSCs seeded onto BGS in vitro initially express pro-angiogenic factors at one wk and based on published studies we expect full expression of pro-osteogenic markers by wk 3. These data may show a dual role for this cell type in supporting BGS survival—initially for neovascularization then for osteogenesis. The animal studies in progress will help reveal if these findings have the potential to translate into clinical improvements in BGS survival.

TESTOSTERONE-SECRETING TISSUE CONSTRUCTION WITH TISSUE ENGINEERING TECHNIQUE

Presenter: Xiaoyun Wang, MD, PhD
Authors: Wang XY, Xing X, Zhou GD, Liu W, Cao YL

Shanghai Changhai Hospital, The second Military Medical University

Introduction: Leydig cells (LCs) of testes are the major testosterone secreting cells in male and play an important role in maintaining the secondary sex characters. With the increasing of Hypoandrogenisum morbidity, how to reconstruct a functional testosterone secreting tissue becomes a vital issue. This study explored the feasibility to construct testosterone-secreting tissue by tissue engineering technique using LCs or co-cultured testis somatic cells (CTSCs).

Methods: 60 Male Wister rats were used. The LCs was harvested by differential anchorage-dependent method. The CTSCs was obtained by co-culture of mixed testes somatic cells. These two kinds of cells were seeded respectively onto the scaffolds of polyglycolic acid (PGA) to form cell-scaffold constructs. The constructs were either cultured for in vitro construction or implanted into gastrocolic omentum of the castrated rats for in vivo construction. The engineered tissue was harvested at 15 days (in vitro) or 3 months (in vivo) for gross and histology examination. The testosterone levels in culture media or serum of implanted rats were detected regularly to evaluate testosterone secreting function of regenerative tissue.

Results: For in vitro construction, the engineered tissue showed a nice appearance and histological structure at 15 days. Unfortunately, the testosterone in culture media was detected only within 48 hours. For in vivo construction, at 3 months, both LC-PGA and CTSC-PGA constructs formed engineered tissue with normal size and shape. The regenerative tissues showed a good vascularization and histological structure similar to normal testes tissue. The serum testosterone level of all cell-PGA implanted rats was much higher than that of PGA alone implanted rats and a higher serum testosterone level was detected in CTSC group.

Conclusion: These results strongly demonstrated that it was completely feasible to construct testosterone secreting tissue by tissue engineering technique. Furthermore, the CTSCs might serve as a better cell source for functional reconstruction of testosterone secreting tissue.
EFFECT OF PORE SIZE ON OSTEOGENIC DIFFERENTIATION OF MURINE PRE-OSTEOBLASTIC MC3T3-E1 CELLS IN A THREE-DIMENSIONAL PLGA SCAFFOLD

Presenter: Madeleine J. Saran, BS
Authors: Saran MJ, Huang W, Ishida K, Huang CK, Hakimi M, Farnad S, Rudkin GH, Yamaguchi DT, Miller TA

University of California - Los Angeles

Introduction: Previous work has shown that larger pore sizes stimulate osteogenesis in vivo. However, the effect of pore size on osteogenic differentiation in vitro remains controversial. We studied the effect of pore size on in vitro differentiation of MC3T3-E1 cells in a three-dimensional (3-D) PLGA scaffold.

Methods: One million MC3T3-E1 cells were seeded onto 3-D poly(l-lactide-co-glycolide) scaffolds with pore sizes 106-150, 180-212, 250-300, 300-425, and 425-500 μm. Cells were cultured with osteogenic media in the presence or absence of BMP-2 (50 ng/ml). Cells were harvested at 4, 7, and 14 days for RNA extraction. Quantitative real-time RT-PCR was performed to measure mRNA expression of osteogenic and angiogenic marker genes: alkaline phosphotase (ALP), osteocalcin (OCN), bone sialoprotein (BSP), and vascular endothelial growth factor (VEGF).

Results: Within our experimental pore size range, we observed a biphasic pattern of ALP, OCN, BSP, and VEGF mRNA expression. Expression of all four genes was initially high in the smallest pore size of 106-150 at days 4, 7 and 14. An increase in pore size to 180-300 caused as much as a 3-fold decrease in gene expression. There was another increase in gene expression as pore size further increased. The highest expression of all four genes occurred at pore sizes of 425-500 (as much as a 4-fold increase compared to 180-212). BMP-2 stimulated expression of all four genes significantly in all tested pore sizes at all time points. However, the most profound effect of BMP-2 was observed in the largest tested pore size, 425-500 (as much as a 14-fold increase compared to 180-212).

Conclusion: Pore size plays a significant role in osteogenic differentiation of MC3T3-E1 cells in vitro. Pore sizes of 425-500 not only support higher expression of osteogenic and angiogenic marker genes, but also maximize the osteo-inductive effect of BMP-2. This data suggests that in tissue engineering applications where the cells are initially grown in vitro and then implanted in vivo, larger pore sizes might be chosen to allow better differentiation both in vitro and in vivo.

SARAH MC3T3-E1 CELLS IN A THREE-DIMENSIONAL PLGA SCAFFOLD

SYNTHESIS OF A TISSUE ENGINEERED PERIOSTEUM WITH ACELLULAR DERMAL MATRIX AND CULTURED OSTEOPROGENITOR CELLS

Presenter: Bjorn H. Schonmeyr, MD
Authors: Schonmeyr BH, Wong AK, Clavin NW, Fernandez JG, Mehrara BJ

Memorial Sloan Kettering Cancer Center

Introduction: Periosteal grafts can aid in fracture repair and promote healing of bone defects by providing bone progenitor cells and acting as a barrier to scar tissue. Unfortunately, these grafts have many of the same disadvantages as bone grafts (i.e. donor site morbidity and limited donor sites). In these experiments we describe a method of synthesizing a periosteum-like material using acellular human dermis (AlloDerm) and bone progenitor cells.

Methods: 1x10^5 fetal rat calvarial osteoblasts were cultured on AlloDerm, maintained in vitro for 10 days, and pulsed with BrdU to assess cellular proliferation. To evaluate the potential of osteoblasts to differentiate into mature bone forming cells, cells were exposed to BMP-4 and assessed for alkaline phosphatase (ALP) production. The potential for gene manipulation of the constructs was assessed by transfection with adenoviral vectors (LacZ and GFP). To investigate the delivery of cells in vivo, bone progenitor cells expressing GFP were cultured on AlloDerm, implanted around the adductor muscle and evaluated after 1, 3, 7 and 14 days.

Results: Osteoblasts grew readily on the AlloDerm forming a multilayer cellular network. In addition, numerous osteoblasts stained positively for BrdU and ALP thus demonstrating active cellular proliferation and differentiation. Furthermore, adenoviral gene transfer was performed with a high rate of transfection and no toxicity. Bone progenitors delivered in vivo remained within the construct and in the interface between the AlloDerm and muscle for 7 days.

Discussion: We show that osteoblasts can be reproducibly cultured on AlloDerm and that these cells retain their ability to proliferate and differentiate. In addition, we show that osteoblasts cultured on AlloDerm can be easily manipulated using recombinant adenoviruses and that cells delivered by AlloDerm in vivo will remain for at least 7 days. Thus, tissue engineered periosteum may be useful in preventing fibrous tissue ingrowth in bone defects while at the same time delivering bone cell progenitors.
**40A**

**ESTABLISHING A NEW HUMAN FULL-SKIN MODEL FOR IN VITRO STUDIES**

**Presenter:** Frank Jacobsen, PhD  
**Authors:** Jacobsen F, Rittig A, Lam G, Steinau HU, Steinstraesser L  
**BG University Hospital Bergmannsheil, Ruhr University Bochum**

**Introduction:** In vitro studies to investigate human wound healing processes are limited because of the lack of reproducible skin models. For the treatment of impaired wound healing transient cutaneous gene therapy will be a therapeutic option for the future. To analyse the gene delivery mode of action more precisely, a new human skin model was established.

**Methods:** Surgically derived full-thickness skin was degreased and sliced into triangular pieces with 3 cm side length. These pieces were placed into special steel constructions to remain the skin under tension. The skin was cultured in an air-liquid interphase for up to 4 weeks. Samples were taken every week for HE-staining and immunohistochemical characterization (Ki-67 and Caspase-3). After 4 weeks 108 IU (infective units) adenoviral vectors containing the LacZ-transgene, which encodes for the E.coli beta-galactosidase, was topically or intradermally delivered. Transgene expression was luminometrically quantified and localized by the x-gal staining method.

**Results:** It was possible to cultivate the skin samples for more than 4 weeks without any loss of epidermal function. With 8 µg beta-galactosidase/mg total protein amount the gene delivery and transgene expression was successful after intradermal application. The topical application showed no effect at any time-point. Additionally, the immunohistochemical analysis demonstrated Ki-67 positive cells within the epidermis at each time-point whereas Caspase-3 staining showed no increase of apoptosis within the 4 week follow-up.

**Conclusion:** Until today no in vitro full-skin model was described that enables investigations for more than two weeks. While we are now able to analyze a 4 week follow-up we are additionally able to use this human full-skin model as a tool to investigate cutaneous gene delivery processes.

**41A**

**EX-VIVO MANIPULATION OF MICROVASCULAR FREE FLAPS FOR IN VIVO EXPRESSION OF TRANSFECTED PROTEINS**

**Presenter:** Cynthia Hamou, MD  
**Authors:** Hamou C, Chang EI, Gurtner GC  
**Stanford University**

**Introduction:** Many protein-deficient related diseases would benefit from transplantation of autologous genetically corrected cells for in vivo expression of the deficient protein. Our laboratory has described a technique using autologous explanted microcirculatory beds (EMBs) or free flaps as bio-scaffolds for seeding genetically corrected cells via their intact microvasculature. Using reporter proteins, we investigated whether in vivo expression of deficient proteins could be generated with transfected adipose-derived stem cells (MSCs) using this system.

**Method:** Superficial inferior epigastric EMBs (n=10) were explanted from rats and maintained ex vivo in a perfusion bioreactor system. 4.0x10^6 MSCs transfected with a retrovirus expressing two fluorescent proteins (non-secreted Green Fluorescent Protein GFP and secreted gaussia luciferase) were infused via the afferent artery during the prolonged (>12hrs) ex vivo cultivation. Seeded EMBs were replanted and harvested on post-replantation days 3, 14, 21 and 45. Seeding efficiency was determined by FISH analysis and proliferation by BrdU/Ki67 assays. Seeded cell fate and secreted protein level was ascertained using fluorescent microscopy, flow cytometry and In Vivo Imaging System (IVIS).

**Results:** MSCs seeded ex vivo egressed in bulk from the microcirculation and formed BrdU+/Ki67+ proliferative clusters in the perivascular space following re-implantation. Seeded EMBs secreted gaussia luciferase protein in the general circulation up to 45 days following replantation. Seeded MSCs remained localized to the EMB without evidence of re-homing or immunological reaction.

**Conclusion:** Here we demonstrate the long-term replacement of a deficient protein using stem cells and EMBs. The abundance of autologous EMBs, their sustainability ex vivo, and the ability to seed them with large numbers of progenitor cells makes this “inside-out” gene therapy paradigm attractive for treatment of inborn errors of metabolism.
42A
A GENETICALLY ENGINEERED PRO-ANGIOGENIC HUMAN SKIN SUBSTITUTE INCREASES VASCULARITY IN GRAFTED WOUNDS
Presenter: Jennifer A. Murphy, MD
Authors: Murphy JA, Straseski JA, Pirnstill SC, Schaeve KK, Comer A, Allen-Hoffmann BL
University of Wisconsin Hospital & Clinics
Introduction: Delays in vascularization of cultured skin substitutes (CSS) contribute to clinical failure. Our CSS were generated from the spontaneously immortalized NIKS human cell line, a consistent source of non-tumorigenic and pathogen-free progenitor keratinocytes. NIKS were genetically engineered to express transgenes encoding the pro-angiogenic growth factor VEGF-165 (CSS-VEGF) and a form of the transcription factor hypoxia inducible factor alpha lacking the oxygen sensitive domain (CSS-HIF1a). The purpose of this study was to determine whether our pro-angiogenic cell lines increase vascularity in grafted CSS.

Methods: CSS-VEGF, CSS-HIF1a, and control CSS were grafted to the dorsum of nude mice (n=3 per CSS). After 9 days grafts were harvested with the wound bed and fresh frozen. Indirect immunofluorescence for CD31 was performed on sections and section images acquired with fluorescent microscopy. Blood vessel density (% area) was then quantified for each graft. Expression of pro-angiogenic and anti-angiogenic genes was also determined by microarray analysis for each cell line.

Results: Blood vessel density for CSS-VEGF was 10.9+/-.1.6%, CSS-HIF1a=4.9+/-.1.1%, and control CSS=2.9+/-.0.7%. CSS-VEGF had more vascular ingrowth than control CSS (p=0.01). Vessel growth induced by CSS-HIF1a was not statistically different from control CSS (p=0.19). Microarray analysis revealed that the NIKS-VEGF cell line had a 2-fold or greater increase in pro-angiogenic growth factors fibroblast growth factor-1 and vascular endothelial growth factor, and in angiogenic inhibitors interferon alpha and beta. The NIKS-HIF1a cell line had a 2-fold or greater increase in the angiogenesis inhibitor interferon beta.

Conclusion: Wound bed vascularity was increased by CSS-VEGF due to the continuous production of VEGF protein. CSS-HIF1a did not produce increased levels of VEGF or increased vascularity relative to control CSS. Future studies will seek to determine the expression profiles of pro-angiogenic and antiangiogenic genes in genetically engineered CSS which promote vascularization of wound beds.

43A
PRO-ANGIOGENIC PROPERTIES OF GENETICALLY ENGINEERED HUMAN KERATINOCYTES
Presenter: Joely A. Straseski, PhD
Authors: Murphy JA, Straseski JA, Akhtar N, Comer A, Auerbach R, Allen-Hoffmann BL
University of Wisconsin Hospital & Clinics
Introduction: Delays in vascularization of cultured skin substitutes (CSS) contribute to clinical failure. Our CSS were generated from the spontaneously immortalized NIKS human cell line, a consistent source of non-tumorigenic and pathogen-free progenitor keratinocytes. NIKS were genetically engineered to express transgenes encoding the pro-angiogenic growth factor VEGF-165 (NIKS-VEGF) and a form of the transcription factor hypoxia inducible factor alpha lacking the oxygen sensitive domain (NIKS-HIF1a). The purpose of this study was to quantify the angiogenic properties of each cell line.

Methods: To visualize angiogenesis mouse corneal micropocket assays (CMAs) were performed for each cell line, NIKS-VEGF and NIKS-HIF1a (n=67 CMAs, 9 clones, 5-8 CMAs per clone). For each CMA a micropocket was created on the surface of the mouse cornea to accept a polyvinyl sponge soaked in cells. On day 11 the mouse was perfused with FITC-dextran and the cornea harvested. Using fluorescent microscopy and image analysis software, angiogenic properties were quantified for each CMA. Neovascularization area (NA) was scaled to account for differences in corneal size and vessel leakage and ranked. Each CMA was also ranked for NA by direct observation. Leakage was quantified on a 0-3 scale.

Results: Calculated CMA rank agreed well with observed rank, R2=0.96. As expected, the positive control had the highest NA, 59%, and the negative control had the lowest, 0%. NA for NIKS-VEGF=39+/-.7%, and NIKS-HIF1a=17+/-.3%, p=0.08. Vascular leakage for NIKS-VEGF=0.8+/-.02% and NIKS-HIF1a =1.5+/-.02%, p=NS.

Conclusion: The CMA is a useful tool to screen pro-angiogenic cell lines for angiogenic potential. Genetically engineered NIKS cell lines show increased vascularization compared to negative controls. As a result, improved wound healing may be observed with skin substitutes generated from these cell lines. This may improve our understanding of how to vascularize engineered tissue to enhance graft survival in skin substitutes or other engineered tissues.
FUNCTIONAL TISSUE ENGINEERING OF FLEXOR TENDONS: CYCLIC UNIAXIAL STRAIN IMPROVES CELL PROLIFERATION, COLLAGEN PRODUCTION AND MORPHOLOGY IN EPITENON TENOCYTES AND ADIPOSE DERIVED STEM CELLS

Presenter: Jonathan Riboh, BS
Authors: Riboh JC, Chong AK, Pham H, Longaker MT, Jacobs CR, Chang J
Stanford University School of Medicine

Introduction: Flexor tendon injuries are both frequent and devastating; especially in severe trauma, where the amount of tendon lost exceeds the supply of autologous grafts. Tissue engineering of flexor tendons promises to help solve this issue. In this study we addressed two challenges: the reduction of cell propagation time in the lab, and the need for improved strength of engineered constructs. This was achieved by studying the effects of cyclic uniaxial strain on two highly proliferative cell lines: epitendon tenocytes (E) and Adipose derived Stem Cells (ASC).

Methods: E and ASC cells were isolated from flexor digitorum profundus tendons and inguinal fat pads of rabbits, and expanded in culture. Cells were transferred to flexible-based culture plates for 24 hours and then submitted to 8% uniaxial strain at 1 Hz for 4 days. On day 2 and day 4, cell number was assessed by counting propidium iodide labeled nuclei. Total soluble collagen was measured using the Sircol colorimetric assay. Collagen I and III levels were measured using an indirect ELISA kit. Cell morphology was assessed by actin and nuclear staining with visualization under confocal microscopy. Statistical analysis was performed using two-tailed, unpaired student t-tests.

Results: Cyclic strain caused a two-fold increase in cell proliferation in both E and ASC cells on day 4 (p < 0.001). Total collagen production was also increased in both E (2 fold vs. control, p = 0.09 ) and ASC (6 fold vs. control, p = 0.0002). Cyclic strain induced a 50% increase in collagen I secretion on day 2 (p = 0.005) and a 76% increase on day 4 (p = 0.007). Collagen III secretion was unchanged. Strained cells from both cell lines became fusiform, with nuclear elongation and parallel alignment of actin filaments.

Conclusion: In this study we demonstrate that E and ASC cells can be induced to proliferate, secrete collagen matrix and assume the morphology of endogenous tendon cells through the application of cyclic uniaxial strain. This suggests an important role for bioreactors and mechanobiology in tendon tissue engineering.

SOLUBLE FACTORS SECRETED BY MATURE CHONDROCYTES PLAY AN IMPORTANT ROLE IN ARTICULAR CHONDROINDUCTIVE ENVIRONMENT

Presenter: Xia Liu, PhD
Shanghai 9th People’s Hospital, Shanghai Jiao Tong University School of Medicine

Introduction: Lineage commitment of stem cells is partially determined by the tissue specific environment provided by adjacent cells. Studies have demonstrated that articular microenvironment can promote bone marrow stromal cells (BMSCs) to differentiate into chondrocytes. However, the exact mechanism is still unclear. Chondrocyte is the major cell type in articular niche. This study explored the important role of mature chondrocytes in chondroinductive mechanism of articular microenvironment.

Methods: Porcine BMSCs (with GFP-labeling) and articular chondrocytes were mixed and implanted subcutaneously into nude mouse or co-cultured on biodegradable scaffolds for 8 weeks. Chondrogenesis was evaluated by gross view, cell morphology, and cartilage specific matrix staining. To further explore the possible mechanism, BMSCs and chondrocytes were separately co-cultured in a trans-well system, in which soluble factors could pass through the mesh but cells could not. In another experiment, BMSCs and paraformaldehyde treated chondrocytes were mixed and co-cultured in cell-pellets to confirm the induced role of cell-cell contact.

Results: The BMSCs in both co-implantation and co-culture constructs differentiated into cartilage phenotype cells, indicated that chondrocytes could provide enough signals for BMSC chondrogenic differentiation in subcutaneous non-chondrogenesis environment or in vitro. The BMSCs in separate co-culture system could be still induced to form cartilage tissue, indicating that soluble factors secreted by chondrocytes alone could provide enough signals for BMSC chondrogenesis. However, the cell-cell contact co-culture alone showed no obvious chondrogenesis.

Conclusion: All these results implied that chondrocyte is a vital component of articular niche and soluble factors secreted by mature chondrocytes play an important role in articular chondroinductive environment. The co-culture of stem cells with mature cells provides a new way for directing stem cell differentiation and also provides a new in vitro model for studying cell lineage commitment and tissue specific niche.
46A
ADIPOSE DERIVED STEM CELLS: CAN THEY BE USED TO TREAT HEMATOPOIETIC MALIGNANCIES
Presenter: Bret M. Schipper, MD
Authors: Schipper BM, Marra KG, Donnenberg A, Donnenberg V, Surti U, Zeevi A, Agha M, Rubin JP
University Of Pittsburgh

Introduction: Many similarities between adipose derived stem cells (ASC’s) and bone marrow derived stem cells have been described in the literature. Importantly, both sources can give rise to hematopoietic cells. This study aims to determine if migratory bone marrow stem cells repopulate the adipose stem cell pool, or if these stem cell pools exist in two separate compartments. If the latter is true, then autogenous ASC’s might be free of the genetic mutations found in hematopoietic malignancies and possibly be used in place of allogeneic bone marrow transplant. To answer this question we examined tissue from patients with two disease states: 1) Chronic Myeloid Leukemia (CML), and 2) history of bone marrow transplant (BMT).

Methods: Adipose biopsies and blood samples were taken from BMT recipients and short tandem repeat (STR) DNA sequences were analyzed to distinguish cells of donor and recipient origin. Adipose biopsies were also taken from patients with CML and evaluated for the presence of the BCR/ABL mutation in ASC’s via FISH and PCR. Only adherent ASC’s were studied to ensure there were no “passenger” hematopoietic cells present. The adipose derived stem cells were then differentiated into hematopoietic cells, as well as into other lineages to demonstrate multipotency.

Results: BMT patients demonstrated 100% engraftment of donor stem cells in peripheral blood samples. However, ASC’s from the same patients were entirely of host origin. In patients with CML testing positive for the BCR/ABL mutation in blood, the ASC’s were free of this mutation by both FISH and PCR. Furthermore, these cells were successfully differentiated into monocytdoid and erythroid colonies.

Conclusion: There appears to be no migration of bone marrow stem cells into the adipose stem cell compartment. This work suggests that the ASC population, while similar in function to bone marrow stem cells, is an independent pool of multipotent cells. This study also suggests that autogenous ASC’s could be used to repopulate the hematopoietic system in the setting of blood malignancies.

47A
PHOTOCHEMICALLY CROSSLINKING COLLAGEN GEL TO IMPROVE SCAFFOLD PROPERTIES FOR ENGINEERING TISSUE
Presenter: Mark A. Randolph, MAS
Authors: Randolph MA, Ibusuki S, Papadopoulos A, Redmond RW, Kochevar IE, Gill TJ
Massachusetts General Hospital

Introduction: Collagen has many favorable attributes as a tissue engineering scaffold in that it is biocompatible, biodegradable and of low immunogenicity. The gel form of collagen is less desirable because it forms only weak hydrophobic bonds when the temperature or pH are raised resulting in mechanically inferior gels. Whereas chemical crosslinking (e.g. glutaraldehyde) strengthens the gel, cells cannot be incorporated. Photochemical crosslinking techniques could strengthen the gel, yet still be cell compatible. The present study was aimed to investigate whether photochemically crosslinking collagen gel can provide a suitable scaffold for chondrocytes to form neocartilage in vivo and whether the neocartilage can integrate with surrounding native matrix.

Methods: Photoencapsulation of isolated swine chondrocytes in collagen type I gel was performed using 0.25 mM riboflavin with exposure to visible light (450-550 nm,40 sec) and evaluated with live-dead assay. As control group, spontaneous collagen gel with seeded chondrocytes was used. Cylindrical constructs of 100 μL were made for the histological and biochemical study of engineered cartilage. A cartilaginous ring model was used to study integration of the neocartilage. All constructs were implanted subcutaneously in nude mice and harvested at 4 and 8 weeks. The samples were examined biochemically, histologically, and with immunostaining for collagen types.

Results: Live-dead assay showed that the cells survived the photoencapsulation process with >90% viability and DNA assay demonstrated the ability of the seeded chondrocytes to proliferate over time. Histologic evaluation of the neotissue revealed morphological and histochemical features characteristic of native cartilage. The glycosaminoglycan content of the neocartilage approximated 71±3.4% of that in native cartilage. Engineered cartilage exhibited integration with adjacent native cartilage.

Conclusion: Photocrosslinking of the collagen gel permits cell survival and improves the qualities of the gel as scaffold for engineering tissues.
**48A**

**ISOLATION AND CHARACTERIZATION OF MESENCHYMAL STEM CELLS DERIVED FROM HUMAN DERMIS**

**Presenter:** Dan Bi, MD  
**Authors:** Bi D, Chen FG, Zhang WJ, Chen FF, Wei X, Zhu L, Liu W, Cui L, Cao

Shanghai Jiao Tong University, School of Medicine

**Introduction:** Although several studies have shown that dermal fibroblasts possess adipogenic, osteogenic, or chondrogenic differentiation potential, no study has characterized this cell population in detail, and there is yet no evidence that a single dermal fibroblast can differentiate into all three cell types.

**Materials and Methods:** Human dermal fibroblasts were isolated from circumcised foreskin by using regular dermal fibroblast culture system. The isolated dermal fibroblasts were also used for clonal analysis by limiting dilution method. Both pooled and cloned fibroblasts were tested for their adipogenic, chondrogenic and osteogenic differentiation potential in respective inductive media.

**Results:** Dermal fibroblasts could be expended in adherent culture for over 40 cell doublings. Cells exhibited adipogenic, osteogenic and chondrogenic phenotypes after respective induction. Among 1000 wells of single cell, 47 clones were established. Clonal analysis showed that 6.4% (3/47) of the clones exhibited three lineages of adipogenic, osteogenic and chondrogenic differentiation potential, while 12.8% (6/47) of the clones exhibited two lineages of adipogenic and osteogenic potential, and 6.4% (3/47) clones exhibited osteogenic and chondrogenic potential. Interestingly, no clone was observed with both adipogenic and chondrogenic potential. Lineage committed clones, including osteogenic (8.5%, 4/47) and adipogenic (2.1%, 1/47) clones, were also observed. However, no chondrogenically committed clone was found. In addition, 63.8% (30/47) of the clones did not show any differentiation potential that had been tested. Furthermore, the tri-potent fibroblasts were Nestin- Vimentin+, which are different from the dermis derived stem cells reported by others.

**Conclusion:** These results indicate that dermal fibroblasts are a heterogeneous population containing progenitors with different levels of differentiation potential, and the Nestin- Vimentin+ fibroblasts may represent a novel type of multipotent adult stem cells in human dermis.

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**49A**

**ECTOTIC BONE FORMATION IN VITRO - ROLE OF LEPTIN ON EXTRACELLULAR MATRIX MINERALIZATION OF CELLS FROM HETEROTOPIC OSSIFICATIONS.**

**Presenter:** Alexander E. Handschin, MD  
**Authors:** Handschin AH, Jung FJ, Wedler V, Wanner GA, Trentz O, Hemmi S, Trentz OA, Giovanioli P

University Hospital of Zürich

**Introduction:** Heterotopic ossification (HO) is the pathologic formation of bone in soft tissue. This process can occur after traumatic brain injury, fractures, and burns. The exact pathomechanism of HO is unknown but probably involves a disturbed bone formation process including osteoblast differentiation. However, to date little is known about the regulatory proteins and cytokines involved in HO formation. Leptin, known as the obesity gene, may regulate normal osteoblast function in vitro. The aim of the present study was to further analyse the pathomechanisms of HO, including a possible effect of leptin in the genesis and regulation of ectopic bone formation.

**Methods:** Primary human osteoblasts (20,000 cells/ml) were cultivated either from normal bone (Group 1) or from resected HO (Group 2). Both groups were incubated with increasing doses of leptin (0, 100, 500, 1000ng/ml). Phenotype expression (RT-PCR: osteocalcin, runx-2) and mineralization of extracellular matrix (alizarin-red, Ca-45 incorporation) were measured after 7, 14 and 21 days.

**Results:** In both groups, osteoblast phenotype expression (osteocalcin, runx-2) was observed, but osteocalcin levels were significantly lower in cells from HO (p<0.05). In both groups, leptin increased the formation of bone nodules in the histological analysis dose-dependent. Extracellular matrix mineralization (Ca-45 incorporation) was significantly increased with 100-1000ng/ml leptin in both groups, with cells from HO showing a significant higher mineralization on day 7 compared to normal osteoblasts (p<0.05). On day 14 and 21, Ca-45 incorporation was similar in both groups.

**Conclusion:** This is the first study to analyse the effect of leptin on bone cells from heterotopic ossification. Similar to the in vitro behaviour of normal osteoblasts, cells from HO respond to leptin exposure with an increased mineralization of the extracellular matrix. In vitro, this response occurs during an earlier stage of cellular differentiation. This mechanism may be involved in the pathogenesis of ectopic bone formation in vivo.
COMPOSITE OSSEOMUSCULO-
CUTANEOUS HEMIFACE/NOSE, LOWER
LIP AND PREMAXILLA ALLOGRAFT
TRANSPLANT

Presenter: Mehmet Bozkurt, MD
Authors: Bozkurt M, Nasir S, Siemionow M
Cleveland Clinic

Introduction: Extensive soft tissue defects of the face are difficult to reconstruct. Composite tissue transplantation could be valuable in facial reconstructive surgery. The most prominent feature of the human face is the nose and unique anatomy of the nose combined with its aesthetic and functional importance makes its reconstruction a challenging. The purpose of this study was to extend application of the face transplantation model in rat by incorporation of the vascularized premaxilla, nose and whiskers transplant.

Methods: In this preliminary study 3 isograft transplants were performed between 3 Lewis (RT1l) rats. The composite nose, premaxilla (including the septum)/whiskers flap was elevated based of the common carotid artery (anterior facial artery) and external jugular vein. Sensorimotor innervation was provided by branch of the facial nerve (zygomatico-facial). At the inguinal region end-to-end anastomosis was performed between common carotid and femoral artery and external jugular and femoral vein using 10-0 nylon. The flap vascularization was studied with gelatin-barium radiographies, showing an intact vascular network after flap harvesting.

Results: All recipients of composite nose, premaxilla and whiskers isografts survived up to 60 days (still under observation). CT scan and bone histology confirmed viability. Pin prick test was performed to evaluate the whiskers function every 2 weeks.

Conclusions: We have introduced a new model of composite osseomusculocutaneous hemiface/nose, lower lip and premaxilla graft transplant. To the best of our knowledge, this is the first time this flap is described. This flap includes sensorimotor units, premaxilla, nose lower lip therefore outcome after transplantation of this flap may provide important information about graft functional recovery.
Saturday, June 23, 2007

Session 8A
Fetal Surgery/Wound Healing
Abstracts 51A – 56A
8:00 – 8:50 am

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51A
TOPICAL TREATMENT WITH MATRIX METALLOPROTEINASE INHIBITOR BATIMASTAT REDUCES WOUND CONTRACTION OF FULL-THICKNESS RAT SKIN WOUNDS
Presenter: Ursula Mirastschijski, MD, PhD
Authors: Mirastschijski U, Claes J, Schnabel R, Agren MS, Schneider W
Otto-von-Guericke University

Background of study: Skin wound healing comprises many phases including wound contraction. Pathological wound contraction can be associated with reduced mobility of joints leading to contractures and immobility of limbs. Myofibroblasts are known to be the main effectors of wound contraction. Matrix metalloproteinases (MMP) contribute to skin wound healing by facilitating keratinocyte migration and tissue remodeling. Intriguingly, MMP-3 knock-out mice lack wound contraction despite unimpaired reepithelialization. Systemic treatment with a synthetic MMP inhibitor reduced contraction of full-thickness skin wounds in mice and rats. Because locally administered MMP inhibitor would minimize the risk of systemic harmful effects of these drugs, the effect on wound contraction was investigated by topical treatment of skin wounds with the synthetic broad-spectrum MMP inhibitor Batimastat.

Methods: Four 8-mm full-thickness skin wounds were made on the back of rats and treated topically either with hydrogel alone (controls) or with the synthetic broad-spectrum, hydroxamate MMP inhibitor Batimastat in hydrogel. Wound area measurements were correlated to reepithelialization assessed by histology and MMP expression measured by gelatin zymography. Immunohistochemistry for alpha smooth muscle actin positive myofibroblasts was performed.

Results: Topical treatment with Batimastat reduced both phases of skin wound contraction significantly compared to controls. On postoperative day 8, wound areas were reduced by only 27% ± 9% (mean ± SD) of the original size compared to 42% ± 16% in controls. Occurrence of myofibroblasts paralleled wound contraction with later onset in MMP inhibitor treated animals. Intriguingly, MMP-9 was increased with Batimastat compared to controls.

Conclusions: Topical application of MMP inhibitor delayed both phases of wound contraction showing an involvement of MMP. These results may prove important for prevention of extensive contraction of unfavorably localized wounds to improve joint mobility and reduce the risk of joint stiffening and patients’ morbidities.

52A
ALTERATIONS IN ENDOTHELIAL CELL BEHAVIOR AND MORPHOLOGY INDUCED BY TENASCIN-C DURING ANGIOGENESIS
Presenter: Henry C. Hsia, MD
Authors: Hsia HC, Schwarzbauer JE
Robert Wood Johnson Medical School/Princeton University

Introduction: Endothelial cell morphogenesis is a critical element of angiogenesis and the wound repair process. Under in vitro three-dimensional (3-D) matrix conditions, endothelial cells can be induced to form a network of capillary-like structures. Using a 3-D fibrin matrix model mimicking the in vivo wound environment’s provisional matrix, the current study examined how this process is affected by alterations in matrix composition through the presence of tenascin-C, a regulatory extracellular matrix (ECM) glycoprotein that is expressed transiently in tissue adjacent to areas of injury and contacts the provisional matrix in vivo.

Methods: Cultured endothelial cells were plated on 3-D fibrin matrices at confluent cell densities and induced to form networks of capillary-like structures by covering the cells with an overlay layer of fibrin matrix in the presence of serum-free media containing insulin, transferrin, and selenium. The effects of different overlay matrix compositions such as the inclusion of tenascin-C were investigated. Cellular morphology and network formation were assessed by light and immunofluorescence microscopy. Protein expression was monitored by immunoblotting techniques.

Results: Within 24 hours, endothelial cells could be observed forming networks of capillary-like structures. The presence of tenascin-C within the overlay layer of fibrin matrix appears to dramatically alter the rate and extent of network formation by endothelial cells. Endothelial cell behavior under these conditions and further matrix effects will be described.

Conclusion: Capillary tube formation on a wound provisional matrix is altered by the presence of tenascin-C. These findings suggest that tenascin-C may play a significant role in influencing in vivo endothelial cell morphogenesis and behavior during wound repair and tissue regeneration.
53A
THE SMAD SIGNALING PATHWAY
ACTION ON OPEN WOUND REPAIR
Presenter: Katherine Au, MD
Authors: Au K, Saggers GC, Allison G, Ehrlich HP
Penn State Hershey Medical Center

Introduction: Transforming growth factor beta (TGF-B) is a profibrotic growth factor that plays a central role in wound healing and modulates cell functions including fibroblast proliferation and the synthesis of type I collagen. Most of TGF-B’s actions are via the Smad signaling pathway including the transcription of the pro alpha-2 type I collagen chain. SB505124 (SB) is a competitive inhibitor of the ALK5 binding site of TGF-B type II receptors, which inhibits the Smad signaling pathway. Does treatment with SB decrease type I collagen synthesis? Does it affect wound contraction and the maturation of granulation tissue?

Methods: Two paired 2x2 cm open wounds were made on the dorsums of 2 rats and photographed. One wound in each pair was treated topically with 1 mM SB and the other served as a control. The wounds were treated for 4 days starting on post-wounding day 2. On day 14, the animals were sacrificed and the wound was photographed and harvested for histologic and biochemical analysis.

Results: There was no significant difference in wound area between treated and control groups. H&E revealed decreased connective tissue deposition in treated wounds. Sirius red stained sections viewed with polarized light conferred less collagen deposition in the SB treated group. Immunohistochemistry showed decreased myofibroblast density in treated wounds. Western blotting confirmed that 3/4 of the wounds treated with SB showed decreased levels of aSMA compared to the untreated control.

Conclusions: SB inhibited the synthesis of type I collagen without affecting wound contraction. Inhibition of TGF-B induced SMAD signaling decreased the density of myofibroblasts in 3 out of 4 treated wounds, and the degree of wound contraction was not affected. These results suggest that type I collagen and myofibroblasts may not be necessary for wound contraction.

54A
THE EFFECT OF DEL1 ON APOPTOSIS
Presenter: Zhen Wang, MD
Authors: Wang Z, Longaker MT, Yang GP
Stanford University

Introduction: Developing endothelial locus (Del)-1 has been described as an angiogenic factor expressed in endothelial cells during early development. We have previously shown that Del1 is expressed in hypertrophic cartilage during development and bone fracture healing. Del1 null mice showed decreased bony callus formation. A related family member, MFG-E8, has been shown to regulate apoptosis. We hypothesized that the Del1 null phenotype is due to its effect on apoptosis during bone fracture healing.

Methods: Human umbilical vein endothelial cells (HUVECs) were grown in the presence of purified Del1 for 12 and 24 h. RNA was harvested and microarray performed to examine the effect of Del1 on gene expression. Apoptosis in HUVECs and normal human chondrocytes, two major cell types in the fracture callus, was induced by different apoptosis inducers and assayed by trypan blue exclusion, WST-8 and TUNEL staining. Apoptosis related kinase pathways were detected by immunoblotting in HUVECs and chondrocytes, and the role of each pathway tested with kinase-specific inhibitors.

Results: Microarray analysis demonstrated down-regulation of apoptosis-associated genes for HUVECs grown in the presence of Del1. Cell viability assays and TUNEL staining showed that Del1 prevents apoptosis induced by starvation, etoposide or anoikis in HUVECs and chondrocytes, two major cell types in the fracture callus, was induced by different apoptosis inducers and assayed by trypan blue exclusion, WST-8 and TUNEL staining. Apoptosis related kinase pathways were detected by immunoblotting in HUVECs and chondrocytes, and the role of each pathway tested with kinase-specific inhibitors.

Conclusion: Del1 has an anti-apoptotic effect on HUVECs and chondrocytes. This is mediated by the ERK and Akt signaling pathways in attached cell assays, but is mediated only by Akt pathways during anoikis. We propose that Del1 aids fracture healing by preventing apoptosis at the fracture callus.
55A
PROTECTIVE ROLE OF I-NOS INHIBITOR IN PARTIAL THICKNESS BURN WOUND

Presenter: Lars H. Evers, MD  
Authors: Evers LH, Bhavsar D, Rennekampff HO, Potenza B, Dobke M, Angle N, Tenenhaus M

University of California

Introduction: In 2005, we presented a clinical report establishing the role of apoptosis in thermally injured tissue in the zone of stasis. This zone is exposed to oxidative stress resulting from reperfusion injury, particularly after sustaining major partial thickness burns. Reperfusion injury patterns result in predominantly apoptotic cellular death. Nitric oxide (NO) plays a significant role in the initiation of the inflammatory cascade, notably from increased expression of macrophage inducible nitric oxide synthase (i-NOS) and is pertinent to burn pathophysiology. An experimental study was designed to develop an animal model of apoptotic cell injury in the zone of stasis in a mouse deep partial thickness burn wound. This protective effect may be due to modification in NO induced vascular permeability as well as free oxygen radical inhibition. This therapeutic intervention may have clinical application for tissue preservation in the thermally injured as well as IR wound states.

Methods: 40 mice (C57BL/6) were anesthetized and received a 30% total body surface area dorsal scald burn. Control group (n=20) received no interventional medicines besides standard resuscitation with normal saline and analgesic agents. Study group (n=20) received 3 mg/kg i.p. S-methylisothiourea (SMT), a specific i-NOS inhibitor every 12 hours. 10 animals in each group were sacrificed at 24 h and 48 h. Visible burn wound and adjacent tissues in the zone of stasis were biopsied for histological review. TUNEL assay, M30 Cytodeath assay, PARP assay and measurement of apoptotic index were carried out on these specimens.

Results: Mean apoptotic index (AI) for control group were 0.248 (± 0.04 SE) and 0.181 (± 0.02 SE) respectively at 24 and 48 hrs. These AIs are comparable to that seen in our clinical observation obtained from human burn samples. The AI for the i-NOS inhibitor group was 0.147 and 0.141 at 24 and 48 hrs respectively. The difference between the groups is statistically significant (p=0.004). The difference was more pronounced at 24 hr time point.

Conclusions: This murine scald model provides a standardized and reproducible methodology for studying tissue injury, apoptosis as well as possible therapeutic interventions. Our results suggest a protective role for i-NOS inhibition in partial thickness burn wounds. This protective effect may be due to modification in NO induced vascular permeability as well as free oxygen radical inhibition. This therapeutic intervention may have clinical application for tissue preservation in the thermally injured as well as IR wound states.

56A
HYALURONAN METABOLIC GENES HAVE DIFFERENTIAL EXPRESSION IN ADULT AND FETAL DERMAL FIBROBLASTS

Presenter: Ali S. Bari, MD  
Authors: Bari AS, Antony AS, Kong W, Longaker MT, Lorenz HP

Stanford University

Introduction: Poorly understood molecular mechanisms permit fetal skin to regenerate while adult skin forms scars. When compared to adult skin, fetal skin is characterized both by an extracellular matrix rich in hyaluronan (HA) and a differential response to cytokines. HA turnover enzymes, HA Synthase (HAS) and Hyaluronidase (Hyal), regulate HA content and are sensitive to pro-fibrotic cytokine TGF-beta1. We hypothesize that HAS and Hyal gene expression responds to TGF-beta1 differently between adult and fetal dermal fibroblasts.

Methods: Mouse skin fibroblasts were isolated in primary culture from E16.5 fetuses and 3 week old adults. Cells were treated with 10ng/ml TGF-beta1 and RNA was harvested at 0, 1, 2, 4, 8, and 24 hours. Expression of HAS (1-3) and Hyal 2 was analyzed using quantitative real time PCR. Secreted HA was collected at 24 and 48 hours and analyzed by ELISA.

Results: At baseline, HAS 1 expression was 6-fold higher in untreated adult cells and Hyal 2 expression was 1.9 fold higher in untreated fetal cells (p<0.05). TGF-beta1 treatment resulted in a 12.8 fold increase in HAS 1 expression and a 8.8 fold increase in HAS 2 expression with adult fibroblasts but resulted in only a 2.8 fold increase in HAS 1 with fetal cells (p<0.05). Hyal 2 expression was decreased 40% with TGF-beta1 treatment in fetal cells (p<0.05). Adult fibroblasts exhibited sensitivity to TGF-beta1 with a 2-fold increase in secreted HA while fetal cells did not respond (p<0.01).

Conclusion: HA metabolism is differentially regulated in adult and fetal fibroblasts. Our data are the first to show the effect of TGF-beta1 on fetal HA metabolism. Pro-fibrotic TGF-beta1 treatment results in greater synthase expression and HA production in adult cells than less responsive fetal cells. These data suggest that fetal skin phenotypes associated with high HA content may involve a complex regulation of HA synthetic and degradative genes.
Saturday, June 23, 2007

Session 9A
Tissue Engineering
Abstracts 57A – 65A
9:30 – 10:50 am

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MAINTAINANCE OF NEURONAL DIFFERENTIATION OF ADIPOSE-DERIVED STEM CELLS IN LONG TERM CULTURE

**Presenter:** Suraj Kachgal, MS  
**Authors:** Kachgal S, Dhar S, Yoon ES, Evans GR  
University of California – Irvine

**Introduction:** Adipose-derived stem cells (AdSCs) have documented great potential to differentiate into cells of a neural phenotype. These cells provide a great source for autologous transplantation into in vivo models of peripheral nervous system disorders. The present study investigates the efficacy of a new neuronal induction media and whether it can maintain human AdSCs in a differentiated state in vitro for a period of time that would correspond with nerve regeneration in vivo.

**Methods:** Human lipoaspirate was processed by standard methodologies and AdSCs from the product were extracted into culture. AdSCs were briefly expanded in control medium and then subjected to culture in our neural induction media (DE-1) for periods of 1 day, 1, 2, 4, 6, and 8 weeks. Cultures were probed for expression of neural-specific markers: NeuN, nestin, GFAP, vimentin, NSE, trk-A, and MAP2 via immunocytochemistry, RT-PCR, and Western blot.

**Results:** Immunocytochemical staining of the neural-induced cells was positive for the markers GFAP, trk-A, nestin, and NeuN. Western blot analysis revealed expression of early neural markers NSE and NeuN was found in control AdSCs and showed decreasing expression in our neural-induced AdSCs, suggestive of a developing neural phenotype. Expression of the early glial marker vimentin was not present in the control but was expressed in neural-induced AdSCs at day one. Vimentin expression tapered off to zero by week eight while expression of the mature astrocyte marker GFAP expressed from day one to week eight. RT-PCR results indicate that all markers except trk-A are transcribed in control and experimental groups, but Western blot analysis shows not all are translated.

**Conclusion:** We have successfully established a medium which promotes neural differentiation of AdSCs and holds them in the differentiated state for a period of time longer than previously reported. The media was successful in promoting the development of cells of a glial phenotype as shown by expression profiles of vimentin and GFAP.

TISSUE ENGINEERED VASCULAR GRAFT PRODUCED BY SELF-DERIVED CELLS AND ALLOGENIC ACCELLULAR MATRIX: BIOMECHANICAL PROPERTIES’ STUDY

**Presenter:** Tiefang Guo, MD  
**Authors:** Guo TF, Yang D, Han X, Hao C, Ma H, Liu G  
Harbin Medical University

**Introduction:** The purpose of this study was to compare the difference on biomechanical properties of tissue engineered vascular graft(TEVG), acellular matrix and fresh canine carotid in vitro. For the sake of shortening the maturation period of the tissue engineered artery, we have designed new pulsatile bioreactor and used deformation as the basic index for mechanical environment control.

**Methods:** Endothelial cells and Smooth muscle cells were used as seeding cells and acellar matrix was used as scaffold to produced TEVG. TEVGs were implanted as carotid interposition grafts. The grafts were obtained at the 6th month. One-dimensional tensile tests were performed for samples of the above acellular matrix, fresh canine carotid arteries and TEVG by Z-K tensile test machine. The bioreactor has been designed as a closed-loop perfusion system. It consists of a processing chamber, a media reservoir, a peristaltic pump, and tubing. There are two systems of media perfusion: one each for inside and outside perfusion of the construct. The control system includes a video camera, a pressure transducer, a solenoid valve, a data acquisition card, and an industrial control computer. A machine vision identifier was developed to accurately control the deformation of the construct during the process of pulsatile perfusion.

**Results:** Tensile test properties of the carotid arteries after decellularization were compared with those of untreated control vessels. Maximum pull and break force of acellular matrix were no lower than those of untreated vessels, as shown in representative curves. But the slope rate of the curve’ midpoint of acellular matrix was bigger than that of untreated vessel, which demonstrated that the compliance of acellular matrix lost a lot. However the compliance of TEVG was similar to untreated vessels. The machine vision identifier recorded the variation of the construct diameter with sufficient space and time resolution, and the circumferential deformation was successfully retained at 10% during the whole perfusion process. Simultaneously, we recorded a pulsatile intraluminal pressure with peak value of 120mmHg.

**Conclusions:** Acellular matrix had well-preserved extracellular matrix and strength sufficient for vascular grafting. This new bioreactor was suitable to mimic shear stress environment and has resulted in the development of constructs with better mechanical properties and morphological characteristics as compared to those created in a static culture medium.
PARACRINE SIGNALING MECHANISMS BETWEEN ADIPOSE- DERIVED STEM CELLS (ASCs) AND ENDOTHELIAL PRECURSORS

Presenter: Luciana Yacomotti, MD
Authors: Yacomotti L, Zuk P, Wasson KL, Bradley JP
University of California - Los Angeles

Introduction: Since adipogenesis and angiogenesis are integrated events, the synthesis of VEGF by Adipose-Derived Stem Cells (ASCs) may contribute to their formation of adipose tissue via stimulation of surrounding endothelial precursors. To initially assess this, the relationship between VEGF, endothelial precursors (EPs) and ASC adipogenesis was investigated.

Methods: ASCs were cultured in three conditions: Group 1: Adipogenic Medium with 10ng recombinant VEGF (VEGF/AM), Group 2: AM with EP-conditioned media (EP/AM) and Group 3: AM with media conditioned by VEGF-treated EPs (VEGF/EP/AM), along with appropriate control conditions. Gene expression of adipogenic markers LPL, PPAR-gamma, ap2 and adiponectin was quantitated by real time PCR.

Results: Group 1: ASCs cultured in VEGF/AM significantly increased their expression of the adipogenic markers after 11 days of induction. However, continued exposure of ASCs to VEGF resulted in a return of these markers to basal levels. Group 2: ASCs cultured in AM with EP-conditioned media (EP/AM) significantly decreased their expression of LPL, PPAR-gamma, ap2 and adiponectin when measured at early and later adipogenic stages (day 11 and day 19). Group 3: A similar decrease was also observed in ASCs cultured with AM with media conditioned by VEGF-treated EPs (VEGF/EP/AM). However, continued differentiation in VEGF/EP/AM (i.e. 19 days) significantly increased the expression of PPAR-gamma and LPL, while returning ap2 and adiponectin expression to basal levels.

Conclusions: The data suggests that ASC adipogenic gene expression can be affected specifically at an early stage of differentiation by stimulation of the ASCs with VEGF. The expression of VEGF by ASCs may affect their differentiation through a signaling pathway that involves surrounding endothelial cells.

CIRCULATING FACTORS EFFECT DIABETIC WOUND HEALING

Presenter: Sandra S. Scherer, MD
Authors: Scherer SS, Pietramaggiori GP, Alperovic MA, Orgill DP, Wagers AJ
Brigham and Women’s Hospital, Joslin Diabetic Center, Harvard Medical School

Introduction: Diabetics suffer from foot ulcers – chronic, non-healing wounds – often requiring lower extremity amputation. The mechanisms by which wound healing is impaired in diabetics remain largely unclear, although cell impairments have been shown. We have hypothesized that blood borne factors, such as cells, can influence diabetic wound healing.

Methods: Wound healing deficient db/db mice were joined in parabiosis to wild-type control mice, such that the two develop a shared blood circulation, with exchange of blood across the vascular junction. Control parabionts consisted of two wild type or two diabetic mice. Both animals in the couples of the parabionts were wounded on the dorsal region with a 0.8 cm2 dermal punch. Wound healing was studied on in these pairs with several criteria, including macroscopic clinical wound closure kinetics, histological, immunhistochemical and immunfluorescence examination.

Results: Diabetic mice joined with wild type mice showed enhanced wound closure (1.4 fold increase), granulation tissue formation (3.9 fold increase), cell proliferation (2.6 fold increase), vascularity (1.6 fold increase) and altered collagen deposition in the wound bed, compared to diabetic control couples. Up to 25% circulating cells were found in the granulation tissue as early as on day 3 and decreased over time.

Conclusions: Circulating cells or factors of a wild type mouse may have functioned to either directly promote wound healing, or to dilute or inhibit negative regulators of wound repair in diabetic animals. This study established a novel model to test circulating cells in wound healing and to further investigate mechanisms involved in diabetic wound healing.
**61A**

**MAGNETICAL SELECTED HUMAN MSCS AND HYDROGEL-BETA TCP/PLGA SCAFFOLDS TO TISSUE ENGINEER HUMAN SHAPED THUMB BONES IN A ONE-STEP PROCEDURE**

**Presenter:** Christian Weinand, MD, PhD  
**Authors:** Weinand CW, Gupta RG, Neville CN, Weinberg EW, Madisch IM, Upton JU, Jupiter JJ, Vacanti JV

Massachusetts General Hospital

**Purpose:** The absence of the thumb, due either to trauma or congenital defect, causes loss of function in daily life that is severely disabling. Recent advancements in tissue engineering, using human mesenchymal stem cells (hMSCs), self assembling hydrogels and 3-D printable scaffolds, have potential to tissue engineer bone. Stem cell populations can be magnetically sorted from bone marrow and differentiated into new bone.

**Methods:** 3-dimensional printed (3DP) ß-TCP /PLGA porous scaffolds were printed according to an ultra-high-resolution volumetric CT (VCT) image of human distal and proximal phalanges. Two hydrogels, a mixture of a new self-assembling hydrogel (PuraMatrix™) and collagen as well as collagen I hydrogel only were prepared. In a single-step procedure human CD 117+ stem-cells were magnetically enriched in hMSCs and were directly mixed in prepared hydrogels and applied onto 3DP ß-TCP /PLGA scaffolds. Constructs were implanted subcutaneously into nude mice for 6 weeks to test ability to form bone. In vivo VCT evaluation, histological evaluation, bone specific gene transcription analysis and biomechanical compression testing were performed at 6 weeks.

**Results:** Radiological evaluation showed densities close to native bone. Histological evaluation using Toluidine blue, von Kossa staining and alkaline phosphatase staining, transcription of bone specific osteonectin and biomechanical testing confirmed formation of new bone. Human origin of newly formed tissue was confirmed by testing for transcription for species specific GAPDH. Samples using the PuraMatrix™/collagen I hydrogel mix had slightly higher mechanical strength and radiological densities than collagen I only. Statistical analysis confirmed high correlations between biomechanical stiffness, densities and bone gene transcription.

**Conclusions:** Bone tissue can be successfully formed in vivo in a single-step procedure using constructs comprised of PuraMatrix /collagen I hydrogel, CD117+ enriched hMSCs and porous ß-TCP/PLGA scaffolds. This new approach could potentially be used in surgical reconstruction for patients with thumb bone loss.

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**62A**

**BIOLOGICAL DIFFERENCES IN ADIPOSE DERIVED STEM CELLS BASED ON AGE AND ANATOMIC LOCATION**

**Presenter:** J. Peter Rubin, MD  
**Authors:** Schipper BM, Marra KG, Rubin JP

University of Pittsburgh

**Introduction:** Adipose tissue has been shown to contain adult stem cells that have therapeutic applications in regenerative medicine. There is evidence that the ability of adipose precursor cells to grow and differentiate varies among fat depots and changes with age. Defining these variations in cell function and molecular mechanisms of adipogenesis will facilitate the development of cell based therapies.

**Methods:** We compared three age ranges and five different subcutaneous adipose depots. Capacity for differentiation of isolated adipose stem cells (ASCs) with and without a strong PPAR-Gamma agonist was examined in-vitro. Cells were also characterized by lipolytic function, proliferation, and sensitivity to apoptosis. Additionally, PPAR-Gamma protein expression was measured.

**Results:** We have observed a difference in the apoptotic susceptibility of ASCs from various depots, with the superficial abdominal depot significantly more resistant. We have also showed that a PPAR Gamma agonist aids in the induction of differentiation in cells from all depots and ages. While sensitivity to apoptosis was linked to anatomic depot, differences in cell proliferation were related primarily to age. Free glycerol production as a result of lipolysis has been shown to be highest in the arm depot. The arm depot has been found to be the only depot to consistently express PPAR Gamma 2 with and without a PPAR Gamma agonist. Also the younger patients have PPAR Gamma expression in all depots, where the older patients only have consistent expression in the arm and thigh depots.

**Conclusion:** There is variability in function of adipose stem cells that have been harvested from different depots. Additionally, we have showed age related changes in function. These data will help select patients and cell harvest sites that best favor tissue engineering therapies.
63A
THE CLINICAL IMPACT OF LONG-TERM TISSUE ENGINEERING BONE BY GENE ENGINEERED STEM CELLS-THE LONG TERM RESULT
Presenter: Sophia Chia-Ning Chang, MD, PhD
Authors: Chang CN, Jeng LB
China Medical University Hospital

Introduction: In repair of large bony defect, autografts, allografts and biocompatible artificial bone substitutes all have their limitation and disadvantages. Tissue engineering may be an alternative methodology for the repairs of bone defects in the clinical setting. However there is lack of long term data to sustain the durability of the regenerated bone.

Objectives: In this study, we test the hypothesis that hBMP-2 transfer to bone marrow stromal cells (MSCs) with collagen type I promotes autologous bone formation for repairing large size craniofacial defect in one year animal model. Methods: The MSCs were isolated from aspired bone marrow from eighteen swine form iliac crest and culture for one month. The MSCs were infected with replication defective adenovirus hBMP-2. Subtotal cranial defects (5cm in diameter) were created, and implanted with MSCs/collagen constructs. After 3 months, 6 months, and 12 months, each 6 swine were sacrificed.

Results: In gross, near-complete repair of cranial defect by MSCs/polymer construct was observed. New bone formation area measured by 3D CT demonstrated significantly improvement after 3months. The maximal thickness of engineered bone is 11mm. Biomechanical test revealed the strength of new bone was similar of normal cranial bone. The Von Kossa staining revealed lamellar bone formation. Adenovirus was absent in all constructs by immunochemical staining proved. There is no significant bone resorption by time, as evidenced by image and biomechanic study.

Conclusions: The data evidenced de novo bone formation capable of sustaining axial compressive loads. Durable repair of large cranial defect in this experiment reveals the efficacy of integration of stem cells, gene therapy and polymer in producing tissue engineered bone.

64A
MECHANICAL ANALYSIS OF A TISSUE-ENGINEERED COMPOSITE FOR OSTEOCHONDRAL RECONSTRUCTION
Presenter: Niamh-Anna A. O’Sullivan, M.D
Authors: O’Sullivan NA, Kobayashi S, Ranka M, Zaleski K, Randolph M, Yaremchuk MJ
Massachusetts General Hospital and Harvard Medical School

Introduction: An inert and biocompatible material could provide an ‘off the shelf’ customizable scaffold offering stability of fixation and integrative repair of small joints secondary to trauma or degenerative disease. We propose a tissue engineered composite construct, combining the biological advantages of tissue engineered cartilage with the mechanical advantages of porous polyethylene (PPE). Adherence and integration of engineered cartilage at the repair site is crucial for stability of the repair. Our aim was to assess the ability of PPE to support the growth of articular cartilage and to assess the biomechanical integrity of the bond formed.

Methods: Porcine articular chondrocytes were isolated and suspended in fibrin gel polymer at 40x10^6 cells/cc and placed between 6mm discs of PPE forming tri-layer constructs. Control samples of fibrin gel or human dermal fibroblasts alone were also made. The constructs were implanted into nude mice for 6, 12, and 18 weeks. Specimens were evaluated for neo-cartilage production and integration into PPE substrate with histological, biochemical, and biomechanical analysis.

Results: Only fibrous tissue formed in the fibrin controls and controls with fibroblasts. New cartilage matrix was formed in all samples with chondrocytes and the neocartilage integrated with the PPE. Integration strength increased over time. Similar trends were observed in failure energy and tensile modulus. Ultimate tensile strength values for experimental samples were increased (p<0.05) from 6 to 18 weeks and exceeded those of controls (p<0.05).

Conclusion: Articular chondrocytes can produce new cartilaginous matrix in vivo that forms mechanically functional bonds with PPE. These findings may have positive implications for tissue engineered articular cartilage for joint resurfacing and reconstruction in the upper extremity.
OXYSTEROLS ENHANCE DIFFERENTIATION OF MURINE PRE-OSTEOBLASTIC MC3T3-E1 CELLS CULTURED ON 3-D PLGA SCAFFOLDS

Presenter: Michael Hakimi, BS
Authors: Hakimi M, Saran MJ, Huang W, Huang CK, Ishida K, Parhami F, Rudkin GH, Yamaguchi DT, Miller

David Geffen School of Medicine at University of California - Los Angeles

Introduction: Although recombinant human BMP-2 has been shown highly effective in the repair of bony defects, it is prohibitively expensive due to the large amount required in clinical applications. In search of a more cost-effective alternative, we studied the osteo-inductive effect of oxysterols, the oxygenated derivatives of cholesterol.

Methods: MC3T3-E1 cells were seeded on 2-D PLGA (poly-L-lactide-co-glycolide) films and 3-D PLGA scaffolds and treated with either 22 (R) Hydroxycholesterol or a 1:1:1 mixture of 22 (R), 22 (S), and 20 (alpha) isomers for 4 days. Cells were harvested after 1, 2, 4, 7, 10, and 14 days of treatment with osteogenic differentiation media. Quantitative real-time RT-PCR was performed to measure mRNA expression of osteogenic markers.

Results: A 4-day pulse-treatment of MC3T3-E1 cells by oxysterols resulted in an equivalent amount of differentiation compared to a 14-day continuous treatment. Treatment of cells on 2-D films by isomer 22 (R) resulted in 6-, 5- and 3-fold increases in mRNA expression of alkaline phosphatase (ALP), bone sialoprotein (BSP), and osteocalcin (OCN), respectively. However, it failed to induce a significant increase in expression of neither gene in 3-D scaffolds. An equal ratio combination of all three isomers resulted in an opposite effect. It was able to induce 31-, 18- and 5-fold increases in expression of ALP, BSP, and OCN, respectively, in 3-D scaffolds. However, its effect on gene expression on 2-D cultured cells was largely inhibitory as determined by expression of ALP and OCN at 14 days. Thus the mixture of oxysterols preferentially accelerates differentiation in cells cultured on 3-D scaffolds, while isomer 22 (R) is preferred on 2-D cultures.

Conclusion: Pulse-treatment of pre-osteoblastic cells by oxysterols enhances osteogenic differentiation. 22 (R) Hydroxycholesterol is sufficient to induce osteogenesis in 2-D PLGA film but all three isomers are necessary in 3-D scaffolds. Oxysterols may represent a potent and cost-effective substitute for BMP-2 in bone tissue engineering.
Saturday, June 23, 2007

Session 10A
Developmental Biology
Abstracts 66A – 71A
11:00 – 11:50 am

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66A
TEMPORARY PARALYSIS OF ABDOMINAL WALL MUSCLES REDUCES THE EVOLVEMENT OF INCISIONAL HERNIA
Presenter: Samuel C. Lien, BS
Authors: Lien SC, Franz MG, Kuzon WM, Urbanchek MG
University of Michigan Medical School

Introduction: The abdominal wall is a muscular structure under a dynamic equilibrium of load forces that are disturbed following laparotomy and during incisional hernia formation. We find that midline laparotomy unloads the transverse and oblique abdominal muscles, causes fiber-type shifts, and decreases abdominal wall compliance. The resultant mechanical force mismatch contributes to the high rate of laparotomy wound disruption and incisional hernia formation. We test the hypothesis that incomplete, bilateral paralysis of the abdominal musculature reduces the size of evolving incisional hernias.

Methods: Using an established rat model, a laparotomy through the linea alba is closed with one mid-incision, fast-absorbing suture promoting central healing while hernias form rostrally and caudally. Three groups (3 x n=6) are compared: a Sham group receives no treatment. The Saline-Hernia (SH) and Botox-Hernia (BH) groups are treated once with equal volume saline or Botulinum Toxin (Botox®, Allergan). On post-operative day 14, incisional hernias and muscle force capacities are measured and adhesions rated.

Results: Hernias developed in all SH and BH but in no Sham rats. When compared to the SH rats, BH group hernias were 42% shorter and 43% narrower (p<0.05). The mechanical force mismatch between RA and EO muscles was apparent in the SH group with stronger RA (+4%) but weaker (-21%) EO muscles compared to the Sham group. RA and EO muscles for the BH group were both weaker than Sham group muscles (-43% and -79% respectively). Both SH and BH rats had more dense adhesions (p<0.05) compared to the Sham rats.

Conclusion: In this incisional hernia model, partial, bilateral paralysis of the abdominal muscles reduces the size of evolving incisional hernias. The mechanical environment for laparotomy repair is improved with temporary paralysis by reducing the mechanical stress across the midline incision and, therefore, increasing compliance. In the BH group, the mechanical force mismatch was reduced thus allowing more complete healing.

67A
MYOFIBROBLAST DIFFERENTIATION AND CONTRACTILE FUNCTION IN IMPAIRED BY HYPOXIA
Presenter: Ali Modarressi, MD
Authors: Modarressi A, Hinz B, Pittet B
University Hospital of Geneva / Ecole Polytechnique Fédérale de Lausanne

Introduction: Ischemia is a powerful promoter of inflammatory reactions at the onset of dermal wound healing. However, continuing hypoxia impairs physiological healing, leading to the formation of chronic wounds with improper wound contraction. Key players in wound contraction are myofibroblasts, specialized fibroblastic cells that exhibit enhanced contractile activity due to the expression of alpha-smooth muscle actin (alpha-SMA). The present study was performed to investigate how different levels of hypoxia influence differentiation, proliferation, survival and contractile function of cultured subcutaneous fibroblasts and myofibroblasts.

Methods: Rat subcutaneous fibroblasts were cultured for 5d either in normoxic conditions (21% O₂), in mild hypoxia (5% O₂) or in severe hypoxia (2.5% O₂) with or without myofibroblast-inducing TGFbeta1. Cell proliferation and myofibroblast differentiation were evaluated by DNA and alpha-SMA staining. Levels of TGFbeta1 were measured with a reporter cell assay. Contraction was assessed by growing (myo)fibroblast cells on deformable silicone substrates.

Results: Compared with normoxic conditions, cell proliferation increased significantly in hypoxia. Both, mild and severe hypoxia decreased myofibroblast contraction, correlating with decreased levels of myofibroblast differentiation. All these effects were independent from the absence or presence of exogenous TGFbeta1. Moreover, levels of active TGFbeta1 measured in 5% and 2.5% oxygen conditions were higher compared with medium in normoxia. However, expression of TGFbeta1 receptor type II were down-regulated in hypoxia.

Conclusion: Our results indicate that hypoxia is an important stimulus for subcutaneous fibroblast proliferation and autocrine production of active TGFbeta1. However, despite high levels of active TGFbeta1 in hypoxia, 5% and 2.5% oxygen levels impair myofibroblast differentiation and contractile function. We are further exploring the molecular mechanisms how hypoxia desensitizes fibroblastic cells against TGFbeta1 with the aim to develop new therapeutic strategies that target altered healing in ischemia.
**68A**

TEMPORAL DIFFERENCES IN FIBROBLAST FUNCTION AND CYTOKINE LEVELS IN ACUTE AND CHRONIC VAC®-TREATED WOUNDS

**Presenter:** Reza Miraliakbari, MD  
**Authors:** Grunfeld R, Roghani R, Natoli N, Hancey J, Hazard S, Noone R, Ehrlich H, Mackay DR, Miraliakbari R  
**Penn State Milton S. Hershey Medical Center**

**Introduction:** The effectiveness of Vacuum Assisted Wound Closure (VAC®) for the treatment of acute and chronic wounds has been clinically established. A unifying understanding of the physiology at a cellular level remains elusive. This preliminary study examines the temporal correlation of cellular function with cytokine levels in acute and chronic VAC®-treated wounds.

**Methods:** Wound fluids were collected from 25 non-diabetic patients treated with VAC® for at least 7 to 10 days. These included 15 chronic wounds (32 collections) and 10 acute wounds (12 collections). Cell migration studies were executed using human dermal fibroblasts. EDA-Fibronectin and collagen type I levels were determined by Dot-Blot and Western blot analysis. ELISA immunoassay measured IL-1 Beta, Vascular Endothelial Growth Factor (VEGF) and MMP-13 (collagenase) levels.

**Results:** In wounds treated with VAC® for over 5 days, acute wounds exhibited significantly greater levels of collagen (p<.01) and VEGF (p<.001) compared to chronic wounds. In contrast, chronic wounds contained significantly greater amount of MMP-13 (p<.05) and an increased cell migration (p = .069) compared to acute wounds. No significant differences between acute and chronic wounds were detected in wound that were treated for less than 5 days with VAC®. In acute wounds, a significant inverse correlation existed between collagen and II-1 levels (p<.001). However, in chronic wounds, a positive correlation existed (p<.001). In addition, in chronic wounds MMP-13 concentration was inversely correlated to fibroblast migration (p<.05).

**Conclusions:** The differential effects of VAC® treatment in non-diabetic acute and chronic wounds were most evident after at least 5 days of VAC® treatment. The progression of wound healing in chronic wounds is signified by lower collagen deposition, lower levels of VEGF, and higher levels of pro-inflammatory cytokines and collagenase. This is in clear contrast to acute wound healing. The correlations highlighted in this study provide further contribution to the study of chronic wound healing.

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**69A**

TWO HUNDRED AND TWENTY-TWO CONSECUTIVE PHARYNGEAL FLAPS: AN ANALYSIS OF POST-OPERATIVE COMPLICATIONS AND SPEECH OUTCOMES

**Presenter:** Patrick Cole, MD  
**Authors:** Cole PD, Banerji S, Boyd V, Hollier LH, Stal S  
**Baylor College of Medicine**

**Background/Objective:** The pharyngeal flap is the most frequently used surgical treatment in the management of velopharyngeal insufficiency; however, recent concerns over poor post-operative outcomes and high complication rates have made the relevance of this procedure increasingly debatable. To evaluate speech outcomes and complication rates following such surgery, a non-randomized retrospective cohort study of pharyngeal flap surgeries was conducted.

**Methods:** A database review was undertaken of all pharyngeal flap surgeries completed from January 2000 to April 2006 at a tertiary, pediatric craniofacial center. Main outcome measures included perceptual speech parameters, nasality, immediate post-operative complications, and obstructive sleep apnea development.

**Results:** Of 222 consecutive pharyngeal flap patients, 150 (68.57%) underwent both pre- and post-operative speech evaluation. Patient mean age at surgery was 6.4 years (range: 3.1 to 17 years). One hundred and twenty-four post-operative patients (82.67%) demonstrated no or mild hypernasality (none: 67.33%, mild: 15.33%). Eight patients (5.33%) presented with mild post-operative hyponasality. Twenty-two patients (14.67%) demonstrated residual VPI. Post-operative complications were rare in this cohort. Twelve patients (8.00%) required supplemental oxygenation for limited desaturations, and 3 patients (1.35%) demonstrated significant post-operative bleeding. Five patients (3.33%) demonstrated positive findings of OSA at 6 months or greater post-pharyngeal flap.

**Conclusions:** Following thorough pre-operative evaluation and planning, the pharyngeal flap can be reliably and safely used to eliminate velopharyngeal insufficiency.
70A
ESTABLISHMENT OF NOVEL HIERARCHICAL CLONOGENIC ASSAY IDENTIFIES UNIQUE POPULATIONS OF ENDOTHELIAL PROGENITOR CELLS

Presenter: Haruchika Masuda, MD
New York University Medical Center

Background: Clinical trials with endothelial progenitor cell (EPC) transplantation to treat ischemic conditions are currently underway. However, there currently exists few functional assay systems of EPCs to help better understand their biology and optimize therapeutic efficacy. The following study aims to establish a novel EPC colony forming assay (ECFA) based on hierarchical EPC clonogenicity.

Methods: Umbilical cord blood-CD133+ cells were isolated by magnetic-bead separation and cultured for 7 days in vasculogenic media and methylcellulose. Colony forming units (CFU) assays were performed to determine cell density, size, and number. EPCs were identified by acLDL uptake, binding UEA-1, and expression of VE-cadherin, eNOS, and VEGF-2 via RT-PCR and cytospin-immunocytochemistry. Tubulization in matrigel and adhesion to an endothelial monolayer were studied.

Results: Two distinct EPC colonies appeared at days 18 to 21; CFU-large and CFU-small EPCs. These colonies differed in cell density (1:70 cell number ratio per colony) and cell size (10 fold). While both colonies featured acLDL uptake and UEA-1, the endothelial expression profile was more intensive in CFU-large EPCs. CFU-large EPCs demonstrated significantly elevated adhesion (1.6 fold) and tubule formation (16 fold) relative to CFU-small EPCs, suggesting that the phenotype of these cells was consistent with more-committed progenitors. This notion was supported in secondary culture experiments, in which CFU-small EPCs transformed into large EPCs after 4 to 7 days and thus appeared to represent a more primitive population. The addition of VEGF to the ECFA system resulted in enhanced EPC expansion; 1.96 and 2.7 fold increase in CFU-small and -large EPCs, respectively.

Conclusions: This study introduces a novel ECFA, based on the hierarchical clonogenicity of EPCs, which can be used to investigate progenitor cell function and their mediators. Our finding that two distinct populations of EPC CFU colonies exist as (small and large) further suggest that optimal cell therapy with EPCs will be based on unique properties at various stages of EPC differentiation.

71A
INHIBITION OF PROTEIN KINASE C (PKC) BY TAMOXIFEN STIMULATES PROCOLLAGENASE PROTEIN SYNTHESIS

Presenter: Charles Y. Tseng, MD
Authors: Tseng CY, Doong H, Lee RC
University of Chicago Medical Center

Introduction: Calcium antagonists such as verapamil are known to induce procollagenase synthesis in human dermal fibroblasts and are used in the treatment of hypertrophic scars and keloids. We have observed that calcium antagonists can induce procollagenase synthesis in fibroblast cells through the seemingly contradictory release of calcium from internal storage sites by the binding of IP3 to its receptors on the endoplasmic reticulum. Increases in cytosolic calcium ion concentration activate the calcium dependent protein kinase C (PKC) signal transduction pathway. We hypothesize that direct PKC inhibitors such as Tamoxifen will also induce procollagenase protein synthesis.

Methods: Keloid fibroblasts were grown in culture and incubated with Tamoxifen for one hour. The cells were allowed to recover from PKC inhibitor treatment by changing the culture media back to Dulbecco’s modified Eagle Medium (DMEM) for twenty-four hours. In the control set, a PKC activator (SC-9) was used to determine if activated PKC would decrease PKC inhibitor induced procollagenase synthesis. These cells were incubated with SC-9 for one hour and then incubated with Tamoxifen for two hours. Using indirect immunofluorescent techniques, procollagenase was localized inside the cells.

Results: The percentage of cells that expressed procollagenase synthesis was determined by dividing the number of cells exhibiting bright fluorescent spots by the total number of cells examined. In the experimental group (Tamoxifen only), 61.7 + 14.2% of treated cells (n=479) expressed increased procollagenase synthesis. In the control group, pretreatment with SC-9 resulted in 3.9 + 2.9% of control cells (n=417) exhibiting procollagenase synthesis.

Conclusion: We investigated the effect of PKC inhibition on procollagenase synthesis in keloid fibroblasts. We found that treatment of keloid fibroblasts with Tamoxifen, a PKC inhibitor, can induce procollagenase synthesis. PKC inhibitors may have potential clinical application in areas of medicine particularly focused on wound healing, scar formation, and the treatment of hypertrophic scars and keloids.
Thursday, June 21, 2007

Session 1B
Other
Abstracts 1B – 5B
4:00 – 4:40 pm

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PREDICTION OF RECURRENCE BY SENTINEL LYMPH NODE BIOPSY IN MELANOMA: THE YALE EXPERIENCE

Presenter: Aaron J. Berger, PhD
Authors: Berger A, Subtil A, Camp RL, Rimm DL, Ariyan S
Yale University School of Medicine

Objective: Define the role of sentinel lymph node (SLN) biopsy in cutaneous melanoma patients and attempt validation of our proteomic assay for prediction of metastatic potential.

Design/Setting: We have developed a molecular/proteomic assay for the prediction of metastatic progression in primary cutaneous melanoma that functions independently of traditional clinicopathologic variables. Trained on a cohort of patients treated before the era of SLN biopsy, we seek to validate this model on an independent cohort of patients who have received SLN biopsy.

Patients: Between 1996 and 2004, 499 melanoma patients underwent SLN biopsy by the same surgeon (SA).

Main Outcome Measures:
SLN status, recurrence (preliminary), death (preliminary).

Results: 58 (12%) of the 499 patients have at least one positive SLN by histologic measures. At an interim follow-up point (mean: 23 months / range: 21 days – 74 months), 50 (10%) patients have developed metastatic disease and 22 (4.4%) have died from their disease. 36 (72%) of the 50 patients with metastatic disease—and 12 (55%) of the 22 patients who died—had negative SLN status.

Conclusion: Despite the early follow-up period, it is clear that SLN biopsy is not completely accurate in the prediction of distant disease or death. This result is probably indicative of the fact that melanoma can metastasize through multiple routes, including lymphatic, vascular, and direct extension. This cohort of patients will be suitable for validation of our molecular model in the prediction of disease outcome.

DIFFERENTIATION OF ADIPOSE DERIVED STEM CELLS (ASCS) TO THE OSTEOCLASTIC LINEAGE

Presenter: Sunil S. Tholpady, MD PhD
Authors: Tholpady SS, Tholpady A, Petrie CP, Sadik KW, Ogle RA, Botchwey E, Ogle RC
University of Virginia

Introduction: Adipose stem cells (ASCs) are small mesenchymal cells of unknown origin that are capable of differentiating into multiple cells types. These include bone, cartilage, nerve, muscle, and many others. We have recently discovered a novel methodology to differentiate ASCs into osteoclasts, thereby creating a cell traditionally thought to be part of the hematopoietic differentiative pathway from a cell that is of mesodermal origin.

Methods: ASCs were derived from liposuction tissue as previously described. Informed consent was obtained from patients per UVA guidelines. Cells were split into two groups – unselected cells, and CD11b selected cells. ASCs were expanded on tissue culture plastic until a sufficient number were available to perform a differentiative assay. Cells were exposed to osteoclastic media (OCM) consisting of DMEM/10% FBS with RANKL. After two weeks, cells were assayed for differentiation by TRAP assay as well as by immunohistochemistry for osteoclastic markers.

Results: Differentiation of ASCs to osteoclasts required OCM exposure for 2 weeks. Cells fused at one week and started to form giant multinucleated cells at this point. However, they did not express markers for osteoclasts, nor did they have TRAP activity. By two weeks, cells were morphologically and immunohistochemically reminiscent of osteoclasts.

Conclusion: In this study, we have successfully created osteoclasts from ASCs. The differentiated cells had immunohistochemical markers that are osteoclast specific, as well as osteoclastic activity as demonstrated by TRAP assay. Selection based on CD11b surface marker caused a 4-5 fold increase in the number of osteoclasts created. Interestingly, the ASCs which are thought to be of mesodermal origin, were able to differentiate into a cell type that is hematopoietic in origin. This differentiation demonstrates the potential for ASCs to become cell types that are beyond their developmental origin, and provides a method for the creation of osteoclasts for in vitro investigations.
3B

HYPERBARIC OXYGEN DOES NOT ALTER THE GROWTH OR PROLIFERATION OF HEAD AND NECK SQUAMOUS CANCER CELLS IN VITRO OR IN VIVO

Presenter: John G. Fernandez, MD
Authors: Schonmeyr BH, Wong AK, Fernandez JG, Clavin NW, Mehrara BJ
Memorial Sloan Kettering Cancer Center

Introduction: Hyperbaric oxygen (HBO) is routinely used for wound healing complications and osteoradionecrosis resulting from treatment of head and neck malignancies. Anecdotal reports have demonstrated explosive tumor growth in patients treated with HBO but conflicting results from both in vitro and in vivo studies on the effects of HBO on tumor behavior have followed. The purpose of these experiments was to evaluate the effects of HBO on squamous cell cancer cells in a controlled mouse model.

Methods: Murine squamous cell carcinoma line SCC-VII were plated in vitro or injected to form flank tumors in C3h/Hej adult mice. Cells were divided into 2 groups (n= 10 each). Group 1 was subjected to daily 90 min HBOT dives at 2.1 atm for 8 consecutive days; group 2 served as control. In vitro proliferation was evaluated by the MTT-assay and tumor growth in vivo was measured by tumor weight and volume. DNA synthesis and cellular proliferation in vivo were assessed by BrdU uptake and the level of hypoxia within the tumors was evaluated by the marker pimonidazole. Serum VEGF was measured by ELISA and angiogenesis was assessed using immunostaining.

Results: After 8 days, no difference in cell number (p=0.534) was detected between the two groups in vitro. Similarly, there were no significant difference in tumor volume or tumor mass (p=0.471) after harvest. Quantitation of BrdU uptake was also similar between the two groups (p=0.338). A trend towards significance in the level of pimonidazol staining was noted in the HBO treated groups implying that tumors exposed to HBO were less hypoxic (p=0.057). At day 8, tumor vessel ingrowth was limited and no detectable levels of VEGF were noted in the serum of either group. Finally, no difference in blood vessel counts was noted, although, newly formed tumor vessels were few in number and abnormal in phenotype.

Conclusion: HBO had no effect on cellular proliferation of squamous cell cancer cells both in vitro and in vivo. In addition, although HBO may affect hypoxia within tumors in vivo, it does not seem to correlate with increasing vascularity or VEGF expression.

4B

REVERSAL OF DIABETIC EPC-DYSFUNCTION THROUGH EX VIVO EXPANSION OF EARLY PROGENITOR CELLS

Presenter: Rica Tanaka, MD
New York University Medical Center

Introduction: Endothelial progenitor cell (EPC) dysfunction may impair neovascularization and delay wound healing in diabetes. Recent studies suggest that stem cell-based therapy with EPCs can improve wound healing or cardiac function. Despite ongoing clinical trials with autologous progenitor cell therapy to augment neovascularization, the mechanism of EPC dysfunction in diabetes remains unknown.

Methods: Type I diabetes(DM) was induced in C57Bl6J by i.p. streptozotocin (stz) injection for 8 days. After 4 weeks of hyperglycemia (>350mg/dl), bone marrow (BM) cells from stz- and wild type mice were magnetically sorted with progenitor cell markers (sca1 and ckit). The number of EPC subpopulations, known as KSL cells (Lin-Sca-1+c-kit+), was assessed by FACS. EPC culture- and vasculogenic colony-assays were performed on freshly isolated KSL cells or cells cultured in serum-free endothelial media (Flt-2, TPO, VEGF, SCF, and IL-6).

Results: The number of KSL cells isolated from BM did not significantly differ between wild type and DM mice (2.8%3± vs 2.8%±3; 1.5 x10⁵ ±1 vs 1.6 x10⁵±1). KSL cells from DM mice produced significantly fewer EPCs in the culture assay (20±2 vs 30±5; p<0.05). The number of large-EPC colonies in our vasculogenic assay was also significantly decreased in DM (2±2.5 vs 6±3; p<0.005), thus suggesting an impaired ability to differentiate into the later-stages of EPC development. Application of our ex-vivo expansion medium to KSL cells led to a significant increase in total- and large-vascular colonies in DM, but not control, samples (10±2 vs 2±2; p<0.001).

Conclusion: This study suggests that EPC dysfunction in diabetes may originate from impairment of EPC-populations to differentiate into mature vasculature. Additionally, we demonstrate the ability to reverse diabetic EPC dysfunction, and achieve supra-physiologic function and number by culturing these cells in serum-free expansion media. Therefore, ex vivo expansion of diabetic EPCs prior to their delivery may represent a more effective approach to therapeutically augmenting wound healing in diabetic patients.
CHRONIC HYPERGLYCEMIA IMPAIRS
HIF-1 ALPHA FUNCTION VIA POST-
TRANSLATION MODIFICATION
PROCESSES IN MOUSE EMBRYONIC
FIBROBLASTS

Presenter: Yubin Shi, PhD, DDS
Authors: Shi Y, Gurtner G
Stanford University

Introduction: Diabetes is a major risk factor for the pathogenesis of macrovascular complications by a reduction of circulating endothelial progenitor cells (EPC). HIF-1alpha is responsible for EPC mobilization, trafficking and proliferation through transcriptional regulation of VEGF and SDF-1, and its function is mainly regulated by post-translation modification. The impaired HIF-1alpha function may lead to the defective vasculogenesis in response to ischemia during chronic hyperglycemia.

Methods: Mouse Embryonic Fibroblasts (MEFs) were cultured in either low or high glucose medium under normoxia or hypoxia (1% O₂). HIF-1alpha expression, DNA binding, trans-activity and protein interaction were measured using Western blot, gel shift/binding activity, HRE-luciferase reporter and immunoprecipitation assays. HIF-1alpha phosphorylation and hydroxylation were confirmed by phosphotase digestion and oxygen-dependent hydroxylase (FIH-1) activity. Reactive oxygen species (ROS) was measured by intracellular detect reagent and hydrogen peroxidase assay.

Results: Although HIF-1alpha protein was accumulated in both low and high glucose cultures during hypoxia, it was de-phosphorylated and partially lost DNA binding activity in high glucose. HIF-1alpha trans-activity in high glucose was decreased by 74% in a time-dependent manner. ROS of MEFs in high glucose was 2.1 folds higher than in low glucose, and was attenuated by deferoxamine. FIH-1 protein level, but not mRNA, was up-regulated 1.9 folds by high glucose. FIH-1 inhibitor DMOG partially rescued HIF-1alpha dysfunction induced by high glucose. The asparaginyl hyper-hydroxylation of HIF-1alpha by FIH-1 in high glucose culture was confirmed by the de-association between HIF-1alpha and p300.

Conclusion: Our data showed HIF-1alpha dysfunction by chronic hyperglycemia resulted from HIF-1alpha de-phosphorylation and hyper-hydroxylation. The de-phosphorylation decreased HIF-1alpha DNA binding; and FIH-1 hyper-activity inhibited HIF-1alpha and p300 association. ROS accumulation by chronic hyperglycemia was probably responsible for HIF-1alpha post-translation modification.
Thursday, June 21, 2007

Session 2B
Tissue Engineering
Abstracts 6B – 11B
5:00 – 5:50 pm

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**6B**

**SIZE LIMITS IN AUTOLOGOUS CELL-BASED ECTOPIC PREFABRATION OF ENGINEERED BONE FLAPS IN RABBITS**

**Presenter:** Oliver Scheufler, MD  
**Authors:** Scheufer O, Schäfer DJ, Jaquiery C, Braccini A, Wendt DJ, Galli R, Gasser JA, Ingold P, Pierer, Heberer M, Martin I

**University Hospital Basel**

**Introduction:** Autologous bone flaps are the gold standard in reconstruction of large bone defects but limited by availability and donor site morbidity. We generated large ectopic bone flaps in rabbits combining flap prefabrication and bone tissue engineering concepts and aimed to determine size limits regarding tissue ingrowth and bone formation within the flaps.

**Methods:** Porous hydroxyapatite scaffolds were fabricated in the shape of tapered cylinders (30mm height, 20mm upper base diameter, 10mm lower base diameter) in order to determine tissue ingrowth and bone formation at different construct diameters. Expanded bone marrow stromal cells (BMSC) from 12 NZW rabbits were uniformly seeded in tapered cylinders at a density of 10 mio. cells per cubic centimeter of scaffold using a perfusion bioreactor. In each animal, two tapered cylinders loaded with autologous BMSC, wrapped in a panniculus carnosus flap and covered by a semipermeable membrane and inserted under the panniculus carnosus (vascularized condition) or covered by a semipermeable membrane and inserted under the panniculus carnosus (non-vascularized condition) were implanted on opposite sides. Constructs were removed after 8 weeks and 12 weeks in 6 animals each and assessed by MRI, µ CT and histology.

**Results:** Vascularized constructs were filled with connective tissue in the outer 3.4±0.6 mm and contained bone in the outer 1.2±0.5 mm, whereas no connective or bone tissue formed in non-vascularized constructs, resulting in significant differences in all histomorphometric parameters. Connective tissue and bone ingrowth was significantly deeper in vascularized constructs after 12 weeks compared to 8 weeks of implantation, indicating progressive centripetal tissue formation.

**Conclusions:** A panniculus carnosus flap supported ectopic prefabrication of large engineered bone flaps in rabbits. However, bone formation was restricted to the outer region of the flaps indicating insufficient vascularization of the central core of the constructs upon implantation. Therefore, advanced strategies are needed to improve vascularization and to overcome the size limits in engineered bone flaps.

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**7B**

**CREATING THICK LINEAR SCAR BY INSERTING A GELATIN SPONGE IN RAT EXCISIONAL WOUND**

**Presenter:** Wei Liu, MD, PhD  
**Authors:** Wu XL, Gao Z, Chua C, Deng D, Cao YL, Liu W

Shanghai Jiao Tong University, School of Medicine

**Introduction:** Rat incisional wound is an important model for wound scarring research, but is also difficult to mimic thick human incisional scarring. We hypothesized that such a thick linear scarring can be generated by inserting a gelatin sponge into a rat excisional wound.

**Materials and Methods:** Total 45 SD rats were divided into three groups. In the first group of 15 rats, an incisional wound of 1cm long was created on the left side of rat dorsal skin as a control. An excisional wound (1 by 0.2 cm) was created on the right side followed by the insertion of a gelatin sponge of (1 by 0.3 by 0.5cm) as an experimental wound. In the second group, 2 excisional wounds with sponge insertion were created in each of the SD rats (n=15). In the third group, 2 excisional wounds (1 by 0.2cm) were created in each of the SD rats (n=15), a gelatin sponge was inserted only in right side wounds. Rats of all three groups were sacrificed at days 7 (n=5), 14(n=5) and 21(n=5) to harvest wound tissues for gross and histological examination.

**Results:** The results demonstrated that the new wound model could generate 11 times wider wound width (at day 7) and 4-5 times wider scar width (at days 14, 21 and 60) respectively than the widths of regular incisional wounds (p<0.05) in tested 15 rats of the first group. The created thick linear scar was grossly apparent in contrast to grossly unobvious scar of the incisional wound, and a regular linear shape could be achieved with similar scar width along the wound. The mechanism study revealed that delayed wound healing, enhanced inflammation, increased expression of fibrotic factors and abnormal wound remodeling might contribute to enhanced scarring. There was no difference in the width between two side wounds (p<0.05) of the second group. In addition, irregular shaped wound/scar was observed in non-inserted wounds when comparing regular linear scar of inserted wounds of the third group.

**Conclusion:** The created thick scar model might be used for scar manipulation study, especially for genetic manipulation in which the inserted gelatin may serve as a gene carrier matrix.
PORE SIZE DETERMINES OSTEOGENIC CAPACITY OF PLAGA CONTRACTS IN VIVO

Presenter: Karim Sadik, MD
Authors: Tholpady SS, Tholpady A, Petrie CP, Sadik KW, Ogle RA, Botchwey E, Ogle RC
University of Virginia

Introduction: Bony regeneration of defects acquired through a number of pathologic processes has the potential to be a major clinical challenge for the plastic surgeon. Many methods have been devised for bone replacement, but they all suffer drawbacks. One approach is the creation of a biomaterial that is capable of supporting bony tissue ingrowth. In this study, poly lactic acid co-glycolic acid is used at differing pore sizes to regenerate calvarial critical size defects in the rat skull.

Methods: Eighteen Sprague-Dawley retired breeders were split into three groups. The first group received a cranial defect 8mm in diameter without any other treatment. The next group was implanted with PLAGA discs with a 200µm pore size. The final group was implanted with 600µm pore size discs. CT scans were taken immediately post-operatively, as well as monthly for 3 months. Animals were sacrificed at 3 months and the defects were processed histologically. Bony regeneration was determined microscopically to determine correlation to CT scanning.

Results: All animals survived the surgery as well as the 3 month observation period. Bone formation was greatest in the first 30 days, with a small amount of bone regenerated during the remainder of the experimental time points. Bone formed by either method was disorganized and immature. Bone formation was greater in the 200µm PLAGA constructs rather than the 600µm ones. The average amount of bone healing was 80% in the former group and 20% in the latter.

Conclusion: Regeneration of tissue requires a precisely defined matrix upon which growth can occur. In this study, we have demonstrated that bone formation is more likely to occur in pore sizes that closely approximate cell diameters, rather than larger pore sizes. Two hundred micron pore size constructs have demonstrated themselves to optimally regenerate bone in this calvarial critical size defect model.

IMPROVEMENT OF IN VITRO CARTILAGE REGENERATION IN THE CENTER OF NEWLY DEVELOPED SILK FIBROIN SPONGE

Presenter: Shinichi Terada, MD, PhD
Authors: Terada S, Ohgo K, Asakura T, Nozaki M, Okano T
National Hospital Organization Disaster Medical Center

Introduction: Our previous study showed that regenerated cartilage in polycaprolactone (PCL) or fibrin glue had a hollow center with a solid outer layer because of the low permeability of oxygen and nutrients into the center through the cartilaginous structure. Therefore, the purposes of this study were to produce a three-dimensional sponge structure using silk fibroin, which sheet is permeable to oxygen and glucose, and to regenerate better cartilage from proliferated chondrocytes.

Methods: Silk fibroin lyophilized powder was dissolved in hexafluoroisopropanol (HFIP) and sucrose particles (250~500 µm) were prepared in a cylinder shape (6 mm x 8 mm). Both silk and PCL sponge (n = 5) were created using the solvent casting and particulate leaching technique. Rabbit ear cartilages were harvested and digested in type II collagenase. Isolated chondrocytes were cultured in a growth medium containing 10% FBS. Proliferated chondrocytes were seeded into polymer sponges and differentiated in serum-free medium containing TGF-beta 2 and des(1-3)IGF-I for 1 to 2 months on a shaker.

Results: Regenerated cartilaginous matrices, which were positively stained in type II collagen and proteoglycan antibodies, were deposited in both the fibroin and PCL sponges. However, the hollow center of the fibroin sponge was smaller than that of the PCL sponge. A comparison of the cross-section surface area showed significantly better cartilage regeneration in fibroin (14.8 ±1.8 mm²) than in PCL (7.1 ±1.4 mm²).

Conclusion: We have successfully established the three-dimensional sponge structure using silk fibroin. We concluded that fibroin had superior ability for improving cartilage regeneration in the center of tissue-engineered constructs.
10B
PROMOTING THE VIABILITY OF INJECTED FAT GRAFTS: THE ROLE OF ADIPOSE-DERIVED STEM CELLS AND VEGF
Presenter: James P. Bradley, MD
Authors: Yacomotti L, Zuk P, Wasson KL, Bradley JP
University of California - Los Angeles

Introduction: Autologous fat injections for the filling of soft-tissue defects may resorb as a result of ischemia, limiting their effectiveness. Previous studies have suggested that fat graft retention relies on VEGF-induced neovascularization. To see if fat graft vascularization and viability may be improved through the secretion of VEGF prior to the onset of ischemic damage, we studied the use of Adipose-Derived Stem Cells (ASCs).

Methods: Part I: In vitro cultures of ASCs (study) were compared to mature adipocytes cultures (control) from multiple sources (n=10). VEGF synthesis was measured by ELISA analysis. Part II: Injection of 1x10^6 human ASCs plus mature adipose tissue (ASC/fat grafts-study group) into athymic rats was compared to injection of fat grafts alone (control) at 1, 2, 4 and 8 weeks. Histology, cell viability and fat graft weights were used for analysis.

Results: Part I: ASCs were found to synthesize significant amounts of VEGF when cultured in vitro. Moreover, the amount of VEGF synthesized per ASC was consistent throughout all ASC populations sampled. VEGF synthesis in a mature adipose specimen varied significantly from another mature adipose specimen. In addition, there was significantly less VEGF synthesis compared to ASC cultured cells.

Part II: Injection of ASCs with mature adipose tissue (ASC/fat grafts) into athymic rats was retained better over time when compared to injection of fat grafts alone (1.2-fold increase in harvested tissue weight – ASC/fat graft vs. fat graft alone, 4 weeks). More importantly, the morphology of the tissues resulting from ASC/fat graft injections appeared to be superior to mature fat controls with well-developed vessels in the ASC/fat graft tissues. Specifically, significant macrophage-mediated tissue degradation was evident in the mature fat graft controls after 4 weeks, whereas a well-retained adipose morphology was apparent upon injection of ASCs in combination with adipose tissue.

Conclusion: Our data suggest that the presence of ASCs results in 1) an increased production of VEGF and 2) an increase in fat graft retention.

11B
FIBROBLAST DIFFERENTIATION OF MOUSE BONE MARROW-DERIVED MESENCHYMAL STEM CELLS
Presenter: Basil M. Hantash, MD
Authors: Zhao L, Li S, Lorenz HP, Hantash BM
Stanford University School of Medicine

Introduction: Skin wound healing involves fibroblast secretion of a provisional matrix, a process that begins 7 days post-injury. Although fibroblasts are normally present in the dermis, this delay in activity suggested that progenitors may be recruited from the systemic circulation. Fibroblasts are assumed to be of mesodermal origin and previous studies have established that bone marrow-derived mesenchymal stem cells (BMSCs) traffic to sites of injury. We therefore examined the possibility that BMSCs serve as fibroblast progenitors during skin injury.

Methods: BMSCs were freshly isolated from the femurs of mice, FACS sorted using an anti-Sca1 antibody, and then cultured in an appropriate medium. At passage 3, BMSCs were switched to a low serum media containing no addition, EGF, FGF2, TGF-beta1, TGF-beta3, or combinations thereof for 3 days then assessed for surface expression of the fibroblast specific marker FSP1 by immunofluorescence. These growth factors were chosen based on previous studies that found them at elevated concentrations in skin wounds. Other genes characteristic of fibroblasts were tested by RT-PCR.

Results: Almost all BMSCs stained positively with anti-Sca1 antibody for all passages tested. Under basal conditions, neither mouse keratinocytes nor BMSCs showed FSP1 staining. Positive FSP1 immunofluorescence was detected only in BMSCs treated with the combination of EGF plus TGF-beta1 and localized to the cytosol as expected. PCR analysis revealed increased collagen 3 expression only in cells exposed to EGF plus TGF-beta1. Neither collagen 3 nor FSP1 were upregulated by treatment with EGF or TGF-beta1 alone.

Conclusion: Thus far, definitive identification of the fibroblast differentiation pathway has remained elusive. Our data provide the first direct evidence that BMSCs are the immediate progenitors of fibroblasts. This is supported by the short induction period of 3 days and physiological relevance of EGF and TGF-beta1 in skin wound healing. Future in vivo studies will help confirm these findings.
Friday, June 22, 2007

Session 3B
Tissue Engineering
Abstracts 12B – 20B
7:00 – 8:20 am

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12B
HEMATOPOIETIC CELLS FUSION IN CTA TRANSPANTATION
Presenter: Wioleta Luszczek, PhD
Authors: Luszczek W, Poptic E, Nasir S, Klimczak A, Siemionow M
Cleveland Clinic

Introduction: The aim of this study was to create ex vivo new generation of chimeric cells by donor-recipient cell fusion.

Methods: Bone marrow (BM) cells were isolated from ACI(RT1a) donors and LEW(RT1l) recipients. Following harvesting, donor BM cells were stained with green PKH67-GL whereas; recipient cells were stained with red PKH26-GL dye. ACI(RT1a) and LEW(RT1l) stained BM cells were fused by standard Poly(ethylene glycol) (PEG) method. Ex vivo fused chimeric cells (RT1a/RT1l) were purified by FACS-sorting using double-fluorescent dye. Efficacy of purification was evaluated by immunofluorescence. Clonogenic potential of fused cells was assessed using colony forming units (CFU) assay. Karyotype analysis was performed to confirm polyplody of the fused chimeric cells. Purified and propagated chimeric cells (1.3x10^6–2.0x10^6) were transplanted by direct intraosseous injection to the six naïve LEW recipients (RT1l) to assess migratory potential.

Results: Chimerism in peripheral blood was evaluated by flow cytometry. Immunofluorescence proved the presence of fused donor-recipient chimeric cells characterized by RT1a/RT1l phenotype which morphologically resembled heterokaryon and synkaryon types of cell fusion. Moreover, polyplody of the fused chimeric cells was confirmed by karyotype analysis. The efficacy of differentiation of chimeric cells was established by CFU assay forming 45-60 colonies. At day 7 after transplantation of fused chimeric cells total chimerism in blood ranged 1.2-4.58% and at day 21, 2.54-2.87%. Following transplantation the engraftment and migratory potential of ex vivo fused chimeric cells was confirmed by their presence in bone marrow compartment of recipients at day 21 post-transplant.

Conclusion: Our study confirmed the feasibility of ex vivo creation of chimeric cells. This investigation reports for the first time successful engraftment of fused chimeric cells from fully MHC mismatch donors into naïve recipients. Moreover efficacy of cell fusion was confirmed by chimerism induction. This approach may be applied as a novel cell-based supportive therapy in solid organ transplant.

13B
IN VITRO CARTILAGE ENGINEERING USING ADULT STEM CELLS AND BIODEGRADABLE POLYMERS
Presenter: Guangdong Zhou, MD, PhD
Shanghai 9th People’s Hospital, Shanghai Jiao Tong University School of Medicine

Introduction: Cell source is always the major problem that hampers clinical application of cartilage tissue engineering. Adult mesenchymal stem cells from bone marrow or fat tissue have high proliferative potential and multi-lineage potential and thus become ideal cell sources for cartilage engineering. This study aims to establish a series of methods compliant to in vitro cartilage construction using adult stem cells and biodegradable polymers.

Methods: (1) Bone Marrow Stem Cells (BMSCs): Human or porcine BMSCs were isolated, expanded, and seeded onto a scaffold of polylactic acid coated polyglycolic acid (PLA/PGA). Chondrogenesis of BMSCs was achieved by three different induced systems respectively. A. Induced by growth factors, including TGFbeta1, IGF-I, and dexamethasone. B. Induced by mixed co-culture with chondrocytes on the scaffold. C. Induced by soluble factors secreted by chondrocytes in a trans-well separate co-culture system. (2) Adipose-Derived Stem Cells (ADSCs): Human ADSCs were isolated, expanded, and sorted by MACS with CD105 as a marker. The CD105+, CD105-, and unsorted cells were seeded onto the PGA/PLA scaffolds respectively and induced by above growth factors.

Results: (1) BMSCs in all the induced systems formed cartilage-like tissue, which was confirmed by typical cartilage histological structures, cartilage specific matrix staining, and expressions of cartilage specific gene, indicating that all the above induced systems are feasible and effective for in vitro cartilage engineering of BMSCs. (2) In ADSC studies, the CD105+ cells formed relatively homogenous cartilage-like tissue superior to the unsorted cells while the CD105- cells only showed sporadic cartilage formation, indicating that CD105 sorting can enrich the chondrogenic potential cells.

Conclusion: These results demonstrate that it is completely feasible to construct cartilage tissue in vitro using adult stem cells and biodegradable polymers. The methods established in this study provide critical technical parameters for clinical application of cartilage engineering based on adult stem cells.
14B
ACCELERATING STEM CELL PROLIFERATION BY DOWN REGULATION OF CELL CYCLE REGULATOR P21
Presenter: Magdalena Plasilova, MD, PhD
Authors: Plasilova M, Fernandez JG, Schonmeyr BH, Clavin NW, Mehrara BJ
Memorial Sloan Kettering Cancer Center

Introduction: Tissue engineering is often limited by the number and proliferation rate of stem cells. No effective treatments for accelerating cellular proliferation while maintaining differentiation potential have been identified. In this report, we show that transient down-regulation of a cell cycle regulator (p21) results in significant acceleration of mesenchymal stem cell (MSC) proliferation without alterations in cellular differentiation potential.

Methods: The effect of p21 down-regulation on MSC proliferation was analyzed using siRNA transfection as well as evaluation of MSCs harvested from p21 knockout (KO) mice. For the siRNA approach, 10^6 bone marrow cells harvested from the wild-type mice were electroporated with 1 μM of p21 siRNA. The downregulation of both p21 protein and mRNA was verified by RNA and protein analyses. Changes in cell proliferation and differentiation were assessed using proliferation assays, q-PCR, and histochemical staining.

Results: siRNA treatment of MSCs resulted in >70% decrease in p21 mRNA and >80% decrease in p21 protein expression. As expected, expression of p21 was undetectable in knockout cells. Cells transfected with p21 siRNA grew 2-times faster than control cells for the first 3-4 days after treatment (p<0.01) and returned to normal proliferation rate after 7 days. Similarly, p21 KO MSCs proliferated 3-4 fold faster than wild-type controls, however, this increase in proliferation was maintained. Histologic analysis demonstrated that p21 deficient cells do not undergo spontaneous osteogenic differentiation during accelerated in vitro expansion.

Furthermore both, p21 siRNA treated and KO cells retained the ability to differentiate normally into osteogenic lineages when cultured in osteogenic conditions.

Conclusion: Our work shows for the first time that siRNA-based inhibition of the p21 cell cycle regulatory gene can transiently accelerate proliferation of MSCs in vitro without altering differentiation potential. These results suggest that optimization of the siRNA-based approach may significantly advance the ex vivo expansion of stem cells used in tissues engineering.

15B
BMP-2 DOES NOT INFLUENCE THE OSTEOGENIC FATE OF ADIPOSE DERIVED STEM CELLS
Presenter: Rebekah K. Ashley, BS
Authors: Ashley RK, Wasson KL, Zuk P, Bradley JP, University of California - Los Angeles

Introduction: Recent studies have shown that BMP2, a potent osteogenic growth factor, in combination with Adipose-Derived Stem Cells (ASCs) can heal critical-sized bony defects. However, whether BMP2 induces an osteogenic response in the ASCs or the surrounding host cells remains unknown.

Methods: Part I: To assess the response of ASCs to BMP2, human ASCs were seeded into Collagraft scaffolds treated with 5 μg BMP2 (ASC+BMP2/Collagraft) and implanted into critical-sized femoral defects. Acellular scaffolds treated with BMP2 (BMP2/Collagraft) and untreated ASC scaffolds (ASC/Collagraft) were used as controls. Healing was assessed histologically and quantitated by micro-CT.

Part II: In addition, human ASCs were induced in vitro with Osteogenic Media (OM) supplemented with BMP2 followed by quantitation of osteogenesis. ASC were treated either continuously, or given an initial pulse of BMP2 for 48 hrs or 7 days followed by maintenance in OM. Finally, flow cytometry was used to quantitate BMPRIA and BMPRIB receptor expression on the ASC cell surface.

Results: Part I: Implantation of ASCs did not result in healing of femoral defects but areas of localized bone formation were identified within the scaffold, whereas implantation of ASC+BMP2/Collagraft scaffolds consistently healed these defects. However, defects implanted with BMP2/Collagraft scaffolds also healed, suggesting that it is the presence of BMP2 rather than the ASC population that is directing healing. Consistent with this, no difference in bone formation was measured between ASC+BMP2/Collagraft and BMP2/ Collagraft scaffolds using micro-CT analysis.

Part II: Moreover, in vitro treatment of human ASCs with BMP2 as a short pulse or continuously did not enhance their osteogenic capacity. The inability of BMP2 to stimulate ASC osteogenesis is likely to be attributed to the expression levels of the BMPRIA and BMPRIB as assessed by flow cytometry.

Conclusions: Taken together, the data suggests that ASCs are not responsive to BMP2 induction both in vitro and in vivo. As a result, BMP2 in combination with these stem cells may not be a viable strategy for the bony healing.
16B MODULATION OF ANGIOGENESIS IN THE AV LOOP MODEL BY MEANS OF FIBRIN GEL-IMMOBILIZED ANGIOINDUCTIVE GROWTH FACTORS

Presenter: Ulrich Kneser, MD

University of Erlangen Medical Center

Introduction: The AV loop model harbours great potential for applications in plastic and reconstructive surgery. Vascularisation of a variety of matrix materials as well as generation of different types of functional tissue has been achieved. However, the process of vascularisation has not been modulated by application of angioinductive growth factors yet. Therefore we aimed to stimulate vascularisation in the AV loop model by application of VEGF and FGF.

Methods: 54 AV loops were constructed in the groin of Lewis rats (250 g) between the femoral vessels using contralateral vein grafts. AV-Loops were embedded in fibrin (10 mg/ml fibrinogen, 2 iU/ml thrombin) (group A). In group B the fibrin matrix contained 100 ng/ml VEGF and 100 ng/ml bFGF. Constructs were encased in isolation chambers. Between days 2 and 28 specimens were explanted and subjected to histological, immunohistochemical (CD31) and morphometric evaluation. Results were compared to intravital dynamic micro MRI and (SEM) images of vascular corrosion replicas.

Results: 48 patent loops were included into the study. Between day 10 and 14 there was a significant burst of angiogenic activity in both groups. Direct luminal neovascular sprouting was evident from the vein and the venous graft but not from the arterial segment. MR angiography reliably detected patency of the AV loops. Vascular density and total number of vascular sprouts was significantly increased in group B (fibrin + VEGF/bFGF) at days 14 and 28 in comparison to group A.

Discussion: This is the first report upon successful modulation of angiogenic processes in the AV loop model by means of application of VEGF and bFGF. Vascularisation starts between days 7 and 10 and the vein and venous graft appear to participate more actively in vascularisation than the artery. In the future, tissue generation in the AV loop model might be efficiently initiated and modulated using several types of growth factors. The combination of tissue-specific cells and growth factors may eventually allow growing significant volumes of axially vascularised, functional tissues for application in reconstructive surgery.

17B FOCAL ADHESION KINASE (FAK) REGULATES OSTEOGENESIS AND ADIPOGENESIS THROUGH ERK SIGNALING PATHWAY IN ADIPOSE-DERIVED ADULT STROMAL CELLS (ADAS)

Presenter: Yue Xu, PhD, MD
Authors: Xu Y, Bekerman E, Chiou M, Wagner D, Longaker MT

Stanford University School of Medicine

Introduction: Focal adhesion kinase (FAK) is a member of a family of non-receptor tyrosine kinase proteins that associates with multiple cellular components including several extracellular matrix proteins, and controls the adhesion and fate of mesenchymal cells. Several signal pathways have been described in regulating the functions of FAK. Adipose-derived adult stromal cells (ADAS) are a population of cells that are capable of self-renewing and multi-lineage differentiation. The aim of this study was to investigate the role(s) of FAK in osteogenesis and adipogenesis and the specific signaling pathway(s) involved in the differentiation.

Method: Mice ADAS were harvested and expanded. Osteogenic and adipogenic differentiation were performed in vitro using established protocols. ADAS were treated with different inhibitors which specifically block several pathways of FAK (U0126 blocks ERK; Wortmannin blocks PI3 kinase and PP2 blocks the SRC/FAK signal). Osteogenesis and early osteogenesis were evaluated. Quantitative real time PCR was performed to assess regulations of adipophilin, PPARgamma, Runx-2 and alkaline phosphatase.

Results: Quantitative alkaline phosphatase activity assay and alizarin Red staining demonstrated the inhibition of osteogenesis in vitro by addition of U0126 to the differentiation media. Oil Red O staining also showed diminished adipogenesis in cells with U0126 treatment. However, the addition of the Wortmannin did not have any effect on either adipogenesis or osteogenesis in these cells suggesting that this specific signaling pathway is not associated with the differentiation. Furthermore, real-time RT-PCR results revealed that blocking ERK/FAK signal pathway significantly inhibited adipophilin, an early adipogenic marker and Runx-2, the critical transcription factor for osteogenesis.

Conclusion: FAK plays an important role in regulation of multipotent mesenchymal cells; therefore, modulations of the FAK/ERK kinase pathway affect ADAS osteogenesis and adipogenesis. Further studies will elucidate the possible mechanism(s) involved in this signal transduction.
18B
Tissue-Engineered Blood Vessel Graft Produced by Self-Derived Cells and Allogenic Acellular Matrix: A Functional Performance and Histological Study

Presenter: Daping Yang, MD
Authors: Yang D, Guo TF, Morris SF
Harbin Medical University

Introduction: Current prosthetic alternatives to autologous vascular grafts remain poor in terms of patency and infection risk. Growing biological blood vessels has been proposed as an alternative. In this work, the authors demonstrate a method for producing a tissue-engineered vascular graft (TEVG) with self-derived endothelial cells (ECs), smooth muscle cells (SMCs) and allogenic acellular matrix in vitro. The aim of this study was to find out if the graft is suitable as the carotid artery substitute.

Methods: A canine model was developed for this study. Endothelial and smooth muscle cells were used as seeding cells and acellular acellular matrix was used as scaffold to produce the TEVG. Endothelial and smooth muscle cells from the saphenous vein were harvested by trypsin and collagenase digestion respectively. These isolated cells were cultured and expanded by routine cell culture technique. The common carotid artery was harvested from other fresh dog cadavers and processed by routine cell culture technique. The common carotid artery was seeded with endothelial cells at the inner surface and seeded with smooth muscle cells at the outer surface. It was placed in bioreactors filled DMEM supplemented with growth factors. After 6 weeks, the vessels were harvested from the bioreactors and seeded with endothelial cells at the lumen for another 3 weeks. Finally, the cell-seeded graft was transplanted to the cell-donated dog to substitute part of the native common carotid artery (2 cm in length). All animals were followed up for 6 months. Twenty-four dogs were divided into 3 groups randomly: group A (native artery graft), group B (allogenic acellular matrix scaffold) and group C (acellular acellular matrix coated with endothelial and smooth muscle cells). These grafts were subjected to regular echocardiography at 1, 3, and 6 months postoperatively. Then, the TEVG were harvested for histological evaluation at 6 months after transplantation. The vessels luminal surfaces were observed by electron scanning microscopy.

Results: The TEVG showed good functional performance demonstrated by regular echocardiography at 1, 3, and 6 weeks, compared to that of native arteries. All vascular grafts in group A and C provided patent rates of 100%, however the patency rate of group B was 50%. The TEVG demonstrated high cell density and development of a highly organized structure of ECM. Biochemical analysis revealed cellularity and proteoglycans and increased collagen contents in the group C, analogous to those of native vessels in the group A.

19B
Genetic Markers of Osteogenesis and Angiogenesis Are Altered in Liposuction Fat Derived Mesenchymal Stem Cells When Cultured on Three-Dimensional Scaffolds

Presenter: Catherine K. Huang, MD
Authors: Huang CK, Huang WB, Zuk P, Jarrahy R, Rudkin G, Ishida K, Yamaguchi D, Miller TA
University of California - Los Angeles

Introduction: Liposuction derived mesenchymal stem (PLA) cells have recently been shown to be capable of differentiating into bone. Most studies on osteoblastic growth and differentiation have been conducted in a conventional 2-D culture system but in native bone, osteoblasts are situated in a 3-D configuration. We have previously shown that mouse pre-osteoblast cells and rabbit bone marrow stromal cells have altered gene expression when grown in a 3-D culture compared to 2-D controls. There have been limited studies of PLA behavior in 3-D systems. We studied the influence a 3-D scaffold has on the expression of genes related to osteogenesis and angiogenesis in PLA cells.

Methods: One million PLA cells were seeded onto 2-D PLGA films or in 3-D PLGA scaffolds, incubated in osteogenic medium, and studied for 21 days. Extracted RNA was subjected to quantitative real-time RT-PCR analysis to study expression patterns of genes involved in osteogenesis and angiogenesis.

Results: When an inert 3-D PLGA scaffold was introduced, the pattern and sequence of gene expression changed significantly. At early time points, PLA cells cultured on 3-D scaffolds had increased expression of angiogenic factors interleukin 8 and vascular endothelial growth factor compared to 2-D controls. VEGF expression remained high in 3-D cultures throughout the 21 days while it significantly decreased in 2-D cultures. Osteogenesis markers - alkaline phosphatase, collagen type I, osteocalcin, osteonectin, and osteopontin - were significantly up-regulated in 3-D cultures relative to 2-D controls after 24 hours and persisted throughout the 21 days.

Conclusions: In human PLA cells the introduction of a 3-D scaffold significantly enhances expression of gene markers of angiogenesis and osteogenesis. A clear sequence is observed. On 3-D scaffolds, PLA cells first up-regulate genes involved with vascular in-growth and then those involved in bone formation. These findings suggest that studying osteogenic differentiation in a three dimensional scaffold may provide a better understanding of the molecular mechanisms underlying bone formation.
THE EFFECT OF ADIPOSE DERIVED STEM CELLS ON FLAP VIABILITY AFTER ISCHEMIA REPERFUSION INJURY

Presenter: Cagri A. Uysal, MD
Authors: Uysal CA, Mizuno H, Tobita M, Ogawa R, Hyakusoku H
Nippon Medical School

Introduction: The stem cells are known to have angiogenesis potential in any situation where ischemia is evident. The BSCs were presented to improve viability of random pattern flaps. We have performed an experimental study to find out the effect of adipose derived stem cells (ASCs) on flap viability after ischemia reperfusion injury.

Materials and Methods: The ASCs were gathered from inguinal fat pads of 6 weeks old ICR mouse. After three passage in control medium (DMEM, 10% FBS), the cells were ready to be injected so that every injection included 1x10^7 cells. The cells were labelled with Dil staining before the injection for tracing. Symmetrical cranial based double flaps were elevated with a dimension of 1cm to 5cm in mice. Each flap was injected either ASCs in 1cc phosphate buffered saline (PBS) or 1cc PBS only. Then the flaps were subjected to 6 hours of ischemia and subsequently reperfusion. On the postoperative 7th day, the flap survival area was measured depending on the length of the flaps (n=12).

Results: The viable flap length in the control group was 15.2 ± 3.4 mm whereas the flap length was 24.4 ± 2.9 mm. There was a statistically significant difference between the groups (p<0.05). Histological examination revealed that the number of capillaries per 20 fields under microscope in the control group and the experimental group were 4.50±0.80 and 7.50±0.75 respectively and the average number vascular density has significantly increased in the ASCs group (p<0.05). This finding was confirmed with anti Von Willebrand Factor (factor VIII related antigen) antibody immunohistochemical staining.

Discussion and Conclusion: The ASCs have a direct angiogenesis effect around 30% that is differentiation to the endothelial cells and indirect effect of 70% that is mainly by differentiation to other specialized cells, important in healing cascades and respectively the secretion of growth hormones and cytokines. The ASCs not only improve the survival of the ischemia reperfusion mediated damaged tissue, but also could differentiate into the necessary cells and tissues in the situations despite of the absence of any scaffold.
Friday, June 22, 2007

Session 4B
Microsurgery
Abstracts 21B – 29B
9:00 – 10:20 am

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21B
ISCHEMIA/REPERFUSION-INDUCED APOPTOSIS IN SKELETAL MUSCLE

Presenter: Wei Z. Wang, MD
Authors: Wang WZ, Fang XH, Stephenson LL, Khiabani KT, Zamboni WA
University of Nevada School of Medicine

Necrosis was considered to be the solo mechanism for ischemia/reperfusion (I/R)-induced cell death. Recent evidence from I/R models of heart, liver, kidney and brain indicates that apoptosis might be the initial mode of cell death in the process to ultimate cell death. However, evidence of I/R-induced apoptosis in skeletal muscle is sparse and divided. The purpose for the present study was to determine whether apoptosis contributes to I/R-induced cell death in skeletal muscle. In the present study, for the first time, we introduce flow cytometry analysis with a double staining technique with Annexin V-FITC and propidium iodide (PI) to simultaneously detect and quantify both apoptotic and necrotic cells isolated from skeletal muscle after I/R in vivo. A rat gracilis muscle model was used. After surgical preparation, clamps were applied on vascular pedicle to create 4h of ischemia and released for reperfusion (I/R, n=10). Clamping was omitted in sham I/R rats (sham I/R, n=10). The muscle sample was harvested after 24h of reperfusion and incubated with collagenase IA followed by EDTA and rat’s own serum. Cells were filtered through a sieve and collected by sedimentation. One million cells from each sample were stained by PI or Annexin V-FITC or both. Twenty thousand cells from each sample were scanned and analyzed by flow cytometry.

Results: The percentage of live cells was 44.6±2% in I/R vs. 64.9±3% in sham I/R (P<0.01). The percentage of necrotic cells was 18±1% in I/R vs. 11.6±1% in sham I/R (P<0.01). The percentage of apoptotic cells was 39.8±3% in I/R vs. 26.6±3% in sham I/R (P<0.01). Obviously, I/R-induced apoptosis was significantly higher than I/R-induced necrosis (P<0.01). The percentage of cells positive for both apoptosis and necrosis was 29.1±2% in I/R vs. 17.3±2% in sham I/R (P<0.01).

Conclusion: Our results clearly demonstrated that I/R not only causes cell necrosis, but also accelerates cell apoptosis in skeletal muscle. Apoptosis contributes to more cell death than necrosis in skeletal muscle after 4h of ischemia and 24h of reperfusion.

Acknowledgement: The dedicated help of Shang Shen from Core Lab, Department of Chemistry, UNLV, is highly appreciated.

22B
GRAFT-DERIVED DENDRITIC CELLS PROMOTE SKIN ALLOGRAFT ACCEPTANCE IN HEMIFACIAL ALLOGRAFT TRANSPLANTS

Presenter: Aleksandra Klimczak, PhD
Authors: Klimczak A, Agaoglu G, Unal S, Siemionow M
The Cleveland Clinic

Purpose: Clinical application of composite tissue allograft transplants opened discussion on the restoration of facial deformities by allotransplantation. We have formerly achieved operational tolerance in fully MHC mismatched rat hemifacial transplants under low dose of cyclosporine-A (CsA) monotherapy. The potential of graft-derived dendritic cells (DC) on chimerism induction was tested in hemiface allografts across MHC barrier.

Methods: Twenty-four hemiface transplantations were performed in 4 groups (6 rats each). Rejection controls included semi-allogenic LBN(RT1l+n) (Group-1) and fully-allogenic ACI(RT1a) (Group-2) donors. Group-3 (LBN donors) and Group-4 (ACI donors) received tapered dose of CsA monotherapy. At different time-point (7, 28, 63, 100 days) samples from lymphoid organs and blood were harvested. Flow cytometry monitored donor-specific chimerism (MHC class-I RT1n and RT1a antigens). Mechanism of allograft acceptance was assessed by the presence of donor dendritic cells (DDC) (immunofluorescence) and apoptotic cells (TUNEL technique) within lymphoid organs.

Results: At day 7 post-transplant DDC and donor leukocytes were detected within spleen and lymph nodes of hemiface transplant recipients. During follow-up, the number of donor-origin DC significantly increased within spleen but only single cells were present within lymph nodes. DDC were absent within thymus. Donor-specific chimerism in the peripheral blood of recipients at day 100: LBN recipients 1.4% CD4/RT1n, 0.5% CD8/RT1n and 2.6% CD45RA/RT1n; ACI recipients 16.8% CD4/RT1a, 3.7% CD8/RT1a. Apoptotic cells were detected at day 7 and during entire follow-up period (100 days) in the lymphoid organs of recipients.

Conclusions: CsA monotherapy promoted tolerogenic properties of DDC in hemifacial allograft transplants due to functional stabilization of DDC at the immature state. Migration and engraftment of graft-derived DDC into lymphoid organs of recipient confirmed immunomodulatory function of DDC in skin allograft acceptance. Anergy of T-cells, demonstrated by the presence of apoptotic cells, contributed to long-term hemiface survival.
23B
SALVAGE OF ISCHEMIC SKELETAL MUSCLE FROM ISCHEMIA/REPERFUSION (I/R) INJURY BY PREVENTION OF MITOCHONDRIAL CALCIUM ([Ca2+]i) OVERLOAD

**Introduction:** We previously reported that postconditioning (PostC) by instigation of 4 cycles of 30sec occlusion/reperfusion at the end of 4h of ischemia salvaged pig latissimus dorsi(LD) muscle flaps from I/R injury and PostC involved reduction of muscle [Ca2+]. The aim of this project is to use pharmacologic probes to prove that prevention of [Ca2+] overload effectively salvages ischemic muscle from I/R injury.

**Methods:** Pigs(18-20kg) with bilateral LD muscle flaps were assigned to one control and 3 treatment groups, which underwent PostC of muscle flaps at the end of ischemia, received an intravenous (IV) injection of Cariporide (3mg/kg) 5min before reperfusion, or IV injection of Cyclosporin A (CsA) (10mg/kg) 5min before reperfusion. Cariporide and CsA are known to prevent [Ca2+] overload in cardiac muscle by different mechanisms. All muscle flaps underwent 4h ischemia/48h reperfusion. Muscle biopsies were taken at 1 and 2h of reperfusion. Muscle infarction was assessed by tetrazolium dye after 48h reperfusion.

**Results:** Infarction in control LD muscle flaps was 47±2%(n=8). Muscle infarction was reduced (p<0.05; n=8) in the PostC (22±1%), Cariporide (24±3%) and CsA (28±1%) groups. At 1h reperfusion, muscle [Ca2+] content (nmol/mg mitochondrial protein) was lower (p<0.05; n=8) in the PostC (372±54), Cariporide (370±39) and CsA (318±43) groups than the control (538±41). At 2h reperfusion, muscle ATP content (umol/g tissue) was higher (p<0.05; n=8) in the PostC (20±4), Cariporide (22±5) and CsA (19±3) groups than the control (8±1). Myeloperoxidase (MPO) activity (units/g tissue) was lower (p<0.05; n=8) in PostC (0.81±0.24), Cariporide (0.61±0.22) and CsA (0.93±0.25) groups than the control (2.06±0.25).

**Conclusion:** We report for the first time that prevention of [Ca2+] overload with CsA or Cariporide given at the end of ischemia, mimics PostC in preservation of ATP synthesis, reduction of neutrophil accumulation (MPO activity) in early reperfusion and salvage of ischemic muscle from I/R injury, thus providing insights into the use of these clinical drugs to salvage ischemic muscle from I/R injury in replantation, thrombolysis and compartment syndrome.

24B
THE EFFECT OF HYPERBARIC OXYGEN ON ENOS PROTEIN EXPRESSION IN ISCHEMIA-REPERFUSION INJURY

**Introduction:** Hyperbaric oxygen (HBO) decreases ischemia-reperfusion (IR) injury through a nitric oxide (NO) mechanism that is nitric oxide synthase (NOS) dependent. We have recently demonstrated that eNOS gene expression at the mRNA level is upregulated both locally and systemically with HBO treatment of IR injury. Clinical experience in previous studies, however, has demonstrated that the benefits of HBO are due to a systemic effect as opposed to a local one. The purpose of this study was to determine if the previously seen gene expression translated to an upregulated protein product either systemically or locally.

**Methods:** A gracilis flap was raised in three groups of Wistar rats (n=10/group): 1) SHAM, 2) IR exposed to 4hrs of ischemia and 3) IR-HBO exposed to 4hrs of ischemia and HBO during the last 90min. of ischemia. After 24hrs of reperfusion, samples were harvested from gracilis muscle and pulmonary vessels. These samples were preserved with liquid nitrogen and the protein was quantified by Bradford Assay. Western Blot analysis was performed to detect eNOS protein. Experimental samples were compared as percentage of SHAM and overall protein content. Data are expressed as mean + SEM. ANOVA was used for statistical analysis.

**Results:** There was no significant difference in eNOS protein expression in the IR-HBO vs. the IR group in gracilis muscle. Expression of eNOS protein in the pulmonary vessels of the IR-HBO group was increased >100% vs. the IR group (235.5+46.8 vs. 125.2+14.7, <0.05). Likewise, the total eNOS protein in the pulmonary vessels of the IR-HBO group was significantly increased compared to the IR group (48.0+5.4 (mu)gm vs. 33.6+2.3 (mu)gm, p<0.05).

**Conclusion:** The significant upregulation of eNOS protein expression in the pulmonary vasculature but not in the gracilis muscle suggests a systemic effect that mimics the clinical scenario of HBO treatment. Further work regarding the modulation of gene expression with HBO and the impact of systemic vs. local eNOS protein is warranted.
SECRETONEURIN ACTS AS A NOVEL MEDIATOR OF NEOVASCULARIZATION WITHIN ISCHEMIC TISSUE

Presenter: Oren M. Tepper, MD
New York University Medical Center

Background: The development of therapeutic modalities to augment neovascularization would be clinically valuable for ischemic conditions in plastic surgery. Recently, secretoneurin (SN) was identified as a novel mediator of endothelial progenitor cell recruitment. The current study tests whether hypoxia serves as an important stimulus for this newly identified angiogenic factor.

Methods: Ischemia was created in skin flaps or hindlimbs of mice and SN expression was identified by immunohistochemistry or radio-immuno assay (RIA). A subset of mice received neutralized SN antibody(ab) intraperitoneally(n=10). SN expression was studied by Western blot in myoblasts, HUVECs, and human SMCs cultured in normoxic or hypoxic (1%O2). Additional in vitro blocking experiments were performed with neutralizing ab and siRNA.

Results: Ischemic tissue demonstrated a marked increase in SN expression compared to controls, and RIA of muscle extracts demonstrated increased SN immunoreactivity (15.1±2.2 vs 7.5±1.9; p<0.05). Delivery of SN antibody prevented an increase in perfusion and capillary density (0.6 and 0.5 of controls; p<0.05). Exposure of myoblasts to hypoxia led to an up-regulation of SN at 36 hours of hypoxia (9.2±1.9 fold, p<0.001), and conditioned hypoxic media contained elevated SN levels (48.2±3.5 vs. 28.2±2.4 fmol SN/106 cells; p<0.05). Notably, no such up-regulation occurred in the absence of serum, thus suggesting the role of a soluble factor. The addition of FGF-2, but not other angiogenic cytokines (i.e. VEGF, PDGF), restored hypoxia-driven upregulation of SN mRNA (8.1±1.3 fold increase; p<0.01). Further incubation with FGF-2 neutralizing ab inhibited this hypoxic response (1.9±1.1 vs 7.1±1.3 fold increase; p<0.01). Similar blocking effects were seen in experiments with siRNA against HIF1-alpha (1.0±0.1 vs 8.5±3.2; p<0.05).

Conclusions: The angiogenic cytokine SN is upregulated in ischemic tissue via HIF1-alpha and FGF-2 dependent mechanisms. These findings identify a novel peptide involved in ischemia-induced neovascularization, and may offer a new candidate for therapeutic angiogenesis for plastic surgery problems.

BONE REGENERATION IN A CRITICAL-SIZED RAT CRANIAL DEFECT USING SKELETAL MUSCLE TISSUE TRANSDUCED WITH AN ADENOVIRAL BMP-2 CONSTRUCT

Presenter: Fangjun Liu, MD
Authors: Liu F, Porter RM, Glatt V, Kwong F, Pilapil C, Betz O, Evans CH
Center For Molecular Orthopaedics

Introduction: The repair of cranial defects remains one of the greatest challenges facing craniofacial and plastic surgeons. Gene therapy approaches to bone tissue engineering promise to improve the treatment of osseous defects and diseases. Effective regeneration of bone is likely to require a scaffold containing osteoprogenitor cells that are exposed to an osteogenic stimulus. Because skeletal muscle contains osteoprogenitor cells, we explored the feasibility of an expedited ex vivo approach in which muscle cells were genetically modified in situ to express BMP-2 and transplanted into cranial defects.

Methods: Critical-sized (8 mm) defects were created in the calvaria of Sprague-Dawley rats. These were engrafted with autologous, skeletal muscle discs (n=3) transduced with recombinant adenovirus (serotype 5) encoding the cDNA for human BMP-2. Additional defects were filled with untreated muscle disk (n=4) or were left empty (n=4). Histological and radiographic assessments (microcomputed tomography, or muCT were carried out 8 weeks post-surgery to determine the amount and type of new bone formation and the percentage of new bone filled within the defects.

Results: Histological and muCT analysis demonstrated that bone regeneration was significant at the defect edges and within the defect site when genetically-enhanced muscle grafts were used. In contrast, bone formation was mainly confined to the defect edges with sham controls or with untreated muscle alone. Quantitative analysis of mineralized tissue within the defects (based on muCT) revealed a significant increase in bone formation (at least 2-fold) using genetically-modified muscle tissue group relative to control groups at the 8-week time point.

Conclusion: This study demonstrates that the direct use of a genetically modified muscle graft as an osteogenic patch can lead to mineralized tissue regeneration within a critical-sized, cranial defect model. The use of “intact” muscle for gene enhanced bone regeneration may be more practical than other tissue engineering alternatives requiring ex vivo cell culture and scaffolds.
27B
GENE THERAPY WITH ADENOVIRUS-MEDIATED VEGF ENHANCES SKIN FLAP PREFABRICATION

Presenter: Raffi Gurunluoglu, MD, PhD
Denver Health

Introduction: We investigated the feasibility in rats of enhancing skin-flap prefabrication with subdermal injections of adenovirus-encoding vascular endothelial growth factor (Ad-VEGF).

Methods: The left saphenous vascular pedicle was used as a source for vascular induction. A peninsular abdominal flap (8 x 8 cm) was elevated as distally based on the epigastric vessels on both sides. After the vascular source was tacked underneath the abdominal flap, 34 rats were randomly divided into three groups according to treatment protocol. The implantation site around the pedicle was injected with Ad-VEGF in group I (n = 10), with adenovirus-encoding green fluorescent protein (Ad-GFP) in control group I (n = 14), and with saline in control group II (n = 10). All injections were given subdermally at four points around the implanted vessel by an individual blinded to the treatment protocol. The flap was sutured in its place, and 4 weeks later, an abdominal island flap based solely on the implanted vessels was elevated. The prefabricated island flap was sutured back, and flap viability was evaluated on day 7. Skin specimens were stained with hematoxylin and eosin for histological evaluation. In two rats from each group, microangiography was performed to visualize the vascularity of the prefabricated flaps.

Results: There was a significant increase in survival of prefabricated flaps in the Ad-VEGF group compared to the control groups: Ad-VEGF, 88.9 +/- 6.1% vs. Ad-GFP, 65.6 +/- 9.4% (P < 0.05) and saline, 56.0 +/- 3.4% (P < 0.05). Sections from four prefabricated flaps treated with Ad-GFP revealed multiple sites of shiny deposits of green fluorescent protein around the area of local administration 1 day and 3 weeks after gene therapy. Histological examination done under high-power magnification (x400) with a light microscope revealed increased vascularity and mild inflammation surrounding the implanted vessel in all groups. Microangiographic studies revealed that the vascularization was distributed in a larger area in the prefabricated flaps treated with Ad-VEGF.

Conclusion: The authors demonstrated that adenovirus-mediated VEGF gene therapy increased the survival of prefabricated flaps, suggesting that it may allow prefabrication of larger flaps and have the potential to reduce the time required for flap maturation.

28B
ELECTROPORATION-MEDIATED PLASMID GENE TRANSFER IN RAT INCISIONAL WOUND

Presenter: Zhen Gao, MD
Authors: Gao Z, Wu XL, Song N, Cao YL, Liu W
Shanghai Jiao Tong University School of Medicine

Introduction: Electroporation mediated gene transfer is relatively safe for application, but no report of its application in incisional wound. This study examined electroporation mediated gene transfer in incisional wound as well as wounding effect on electrotransferred gene expression in skin.

Methods: In Sprague-Dawley rats, dorsal skin was electroporated after injection of pVAX1 plasmid DNA (1ug/ul, in 100ul PBS) containing enhanced green fluorescence protein (EGFP) gene. Electroporated skin was also incisionally wounded either immediately after or 24 hours after electroporation. Harvested specimens were frozen sectioned and examined for EGFP expression followed by H&E staining. The expression was also quantitatively analyzed with integrated optical density (IOD).

Results: A protocol of 800 voltages in amplitude with 6 square wave pulses, each lasting 20 milliseconds with 200 millisecond interval was found optimal to mediate EGFP expression in dermis (fibroblasts and hair follicles) and epidermis. Lower or higher voltages resulted in only epidermal expression, similar to plasmid injection without electroporation. Importantly, the high expression could cover a wide area of injected skin or an incisional wound. In dermis, the expression reached the highest level between day 1 and day 2 post-electroporation, remained relatively high at day 4, significantly decreased at day 6 and became non-detectable at day 14. Incisional wounding was found to decrease EGFP expression in about 50 percentage at day 2 post-electroporation comparing with non-wounded skin, the level of wound skin was slightly lower as well at days 4 and 6. Wounding 24 hours after electroporation was found to better maintain gene expression than the wounding made immediately after electroporation. Histologically, electroporation did not affect healing results of incisional wounds.

Conclusion: Electroporation can efficiently mediate gene expression in an incisional wound, although wounding may also affect electrotransferred gene expression.
NARINGENIN INHIBITS NEOINTIMAL HYPERPLASIA FOLLOWING ARTERIAL RECONSTRUCTION WITH INTERPOSITIONAL VEIN GRAFTS

Presenter: Cenk Cayci, M.D.
Authors: Cayci C, Wahlquist TC, Seckin SI, Martens TP, Oz MC, Ascherman JA

Columbia University

Background: Stenosis secondary to neointima formation is a major cause of failure following arterial reconstructions. Vessel wall homeostasis is regulated by proinflammatory cytokines that affect smooth muscle cell proliferation, growth, migration, and death. We assessed the hypothesis that naringenin, a flavinoid possessing anti-inflammatory, anti-oxidant, and anti-proliferative activities, reduces neointimal hyperplasia following vascular injury.

Methods: Arterial injury was created by interposition grafting of an autologous right superficial epigastric vein graft into the right femoral artery in 48 rats. The rats were then divided into four groups of 12. Two groups were treated with naringenin for 2 and 4 weeks each while 2 control groups received normal saline for the same durations. Femoral arteries were studied at the end of the treatment. Thickness of intima was measured, and levels of PDGF-BB, TNF-alpha, and Ki67 labeling index were quantified with immunohistochemistry to assess the amount of neointimal hyperplasia.

Results: Although there were no significant differences between the groups at 2 weeks, neointima thickness was lower in the naringenin treated group at 4 weeks (23.7 ± 2.3 vs. 35.6 ± 2.6 micrometers in control group; <0.001). The levels of PDGF-BB and TNF-alpha were lower in naringenin treated groups at both 2 weeks (0.21 ± 0.03 for PDGF-BB vs. 0.39 ± 0.05% in control group, <0.001), (21.2 ± 0.8 for TNF-alpha vs. 36.1 ± 1.9% in control group, <0.001) and 4 weeks (0.25 ± 0.03 for PDGF-BB vs. 0.57 ± 0.09% in control group, <0.001), (25.5 ± 1.8 for TNF-alpha vs. 45.0 ± 2.9% in control group, <0.001). Ki67 labeling index was lower in naringenin treated groups at 2 weeks (13.9 ± 2.8 vs. 18.7 ± 3.7% in control group, <0.05) and at 4 weeks (17.5 ± 2.6 vs. 31.1 ± 4.7% in control group, <0.001), indicating a lower level of cellular proliferation.

Conclusion: Naringenin reduced neointimal hyperplasia following arterial reconstruction in this rat model. This may be mediated by a decrease in PDGF-BB and TNF-alpha levels, and the resulting down regulation of smooth muscle cell proliferation.
Friday, June 22, 2007

Session 5B
Nerve
Abstracts 30B – 35B
11:00 – 11:50 am

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30B
CORTICAL BRAIN MAPPING OF THE RAT FORELIMB USING FMRI AT 9.4T BY DIRECT NERVE STIMULATION
Presenter: Younghoon R. Cho, MD PhD
Authors: Cho YR, Pawela CP, Li R, Yan JG, Matloub H, Hyde JS
Medical College of Wisconsin

Purpose: Brachial plexus injuries can result in debilitating partial or complete loss of function of the affected upper extremity. Current treatment options are often limited to surgical reinnervation of the injured nerves. Past fMRI studies have been focused on mapping the rodent somatosensory system using direct forepaw stimulation. This study aims to refine our current knowledge of the cortical representation of three major nerves of the upper extremity musculocutaneous, ulnar and median nerves. This is achieved by direct electrical nerve stimulation during fMRI acquisition using a 9.4T scanner. This effort is part of a larger goal of developing a cortical maps for both motor and sensory nerves of the rodent upper extremity and developing a rodent model for brachial plexus injuries and surgical nerve repair.

Methods: 15 Sprague Dawley rats were used to study the musculocutaneous (5 rats), the ulnar (5 rats) and the median (5 rats) nerves. The brachial plexus of each rat was exposed on the right upper arm, and the nerve in question was isolated and secured to a stainless steel electrode. Stimulation protocols differed in current (0.5 mA or 1.0 mA) or frequency (5 Hz or 10 Hz). A bipolar beryllium copper electrode was placed in the left forepaw to serve as a control. Each stimulation sequence lasted 3 minutes 40 seconds and the fMRI scans were acquired on a 9.4T Bruker MRI scanner.

Results and Discussion: Each nerve showed a distinct representation in the motor and sensory regions of the cortex (Figure 1). Increased frequency (10Hz) and current intensity (1.0 mA) showed greater levels of cortical stimulation and sometimes extended to the other cortex. Though the motor and sensory regions of the three nerves can be seen in the slices shown below, there is some overlap in the activation across the nerves. This study provides a refinement of our understanding of the specific motor and somatosensory cortical representations of the brachial plexus. Further studies are warranted to refine the individual sensory and motor inputs of each nerve.

31B
THE EFFECTS OF INFLAMMATION ON GFAP EXPRESSION IN SATELLITE CELLS OF THE DORSAL ROOT GANGLION
Presenter: Krzysztof B. Siemionow, MD
Authors: Siemionow KB, Klimczak A, Siemionow MZ, McLain RF
Cleveland Clinic

Background: Satellite cells (SC) are neuroglial cells that closely interact with nerve cells of the dorsal root ganglion (DRG). The role of SC remains unknown. Glial Fibrillary Acidic Protein (GFAP) is the principal intermediate filament in mature astrocytes. GFAP is thought to be important in astrocyte-neuronal interactions and is believed to modulate astrocyte motility and shape. The objectives of this study were to identify the effects of inflammation on the expression of GFAP in the DRG at various time points.

Methods: Seventy-two rats were used. After a hemilaminectomy was performed, two distinct procedures at the L5 DRG were investigated: 1) Group I, the inflammation group, in which fragments of chromic-gut suture were laid adjacent to the DRG; 2) Group II, tight ligation, the ischemia group, in which the root was tightly ligated proximal to the DRG with chromic-gut. DRGs were harvested at 6hrs, 24hrs, 48hrs, 72hrs, and 7 days post-operatively. The contralateral DRG served as a control. The harvested DRG were analyzed using light microscopy for SC immunoreactivity using GFAP polyclonal antibody. Two observers analyzed the slides and expression of GFAP was scored as: None=0, Mild=1, Moderate=2, Intense=3. The percentage GFAP expression was assessed by counting all SC that express GFAP and dividing them by the total number of SC.

Results: 150 dorsal root ganglia were harvested and available for analysis. Naive controls did not express GFAP. In group I, GFAP expression was observed in SC and SC sheaths and increased from post-operative hour 6 and peaked on post-operative day 3 in both the treated and control groups. Nerve cells did not express GFAP at any time point. Group II, SC did not express GFAP at any time point. However, their internal controls did express mild levels of GFAP in approximately 20% of SC.

Conclusions: We have shown that SC express GFAP in response to inflammation and that this expression increases as the inflammatory process develops. This would support the role of the SC as key components of the inflammatory process and as possible mediators of nerve cell inflammation, repair, and scar formation.
A NOVEL IMMUNOLOGIC DEMYELINATION THERAPY TO ENHANCE NERVE REGENERATION IN THE PERIPHERAL NERVOUS SYSTEM

Presenter: Aaron M. Kosins, BS  
Authors: Kosins AM, Mendoza C, McConnell MP, Shepard B, Dhar S, Evans GR, Keirstead HS

University of California – Irvine

Introduction: In order to improve the regenerative potential of PNS axons in vivo, we utilize a novel therapy in the adult rat sciatic nerve in which nerve regeneration is enhanced following contusion and transection injuries. We demonstrate that 1) Axon regeneration within a region of injury increases in the presence of immunological demyelination, and 2) Regenerated axons are derived from the proximal motor axons. This data will be applied in the creation of tissue-engineered constructs using a combination of cell-based and structural therapies to bridge critical defects in the PNS.

Methods: Adult female Sprague-Dawley sciatic nerves were contused and injected with the demyelinating agent. The sciatic nerves were harvested 14 and 28 days following the onset of demyelination. In a second group, the sciatic nerves were exposed, transected, repaired, and injected with the demyelinating agent. These animals were similarly euthanized and processed to examine the extent of axon regeneration. Specimens were fixed and evaluated using structural and immunohistochemical analysis. Tracers were included to determine the source and direction of axonal re-growth.

Results: A single epineural injection of complement proteins plus antibodies to galactocerebroside (the major myelin sphingolipid) resulted in demyelination followed by Schwann cell remyelination that enhanced nerve regeneration in the injured (contusion and transection) animals. At fourteen days, peripheral nerve regeneration clearly demonstrated spanning of the injured sciatic nerve segment. At twenty-eight days, peripheral nerve regeneration continued to improve. Tracers demonstrated that nerve regeneration arose from proximal motor axons, and not the distal branching of sensory axons.

Conclusion: These studies demonstrate a novel method of enhanced nerve regeneration in the PNS using experimental immunological demyelination.

PREPARATION AND INTEGRATION OF NERVE CONDUITS USING A PHOTOCHEMICAL TECHNIQUE

Presenter: Anne Catherine O’Neill, MD  
Authors: O’Neill AC, Randolph MA, Bujold KE, Kochevar IE, Redmond RW, Winograd JM

Massachusetts General Hospital, Harvard Medical School

Introduction: Recent research has focused on alternative methods of bridging nerve gaps, which would avoid the need for autologous tissue harvest and its consequent donor morbidity. Photochemical tissue bonding (PTB) is a developing tissue repair technique that facilitates water-tight sealing between tissue surfaces. This study aims to determine whether axonal regeneration can be enhanced by photochemical sealing of nerve conduits. We investigate photochemical integration of human amniotic membrane (HAM) and Type I bovine collagen conduits in a rat nerve gap model. We also evaluate PTB as a crosslinking technique to confer structural stability to HAM conduits.

Methods: Conduits were prepared from HAM and collagen sheeting using a photochemical technique. A 1cm gap was created in the sciatic nerve of 40 Sprague-Dawley rats. HAM or collagen conduits were placed across the gap and either secured with sutures or sealed using PTB. The excised nerve segment was reversed and replaced in the control group, representing the current gold standard of autologous nerve grafting. At 12 weeks postoperatively nerves and gastrocnemius muscles were harvested for histology and histomorphometry.

Results: HAM conduits crosslinked with PTB maintained their tubular structure with no cases of conduit collapse in vivo. Regeneration within the HAM conduit sealed with PTB was found to be significantly better than that observed in the amnion conduit/suture group. Regeneration in the HAM/PTB group did not differ significantly from the autologous nerve graft group. In the collagen conduit/PTB group the nerves were not in continuity at harvest indicating that PTB is unsuitable for integration of this conduit. Regeneration occurred in the collagen/suture group but to a significantly lesser extent than the other groups.

Conclusion: PTB sealing improves regeneration within conduits compatible with this tissue repair system. PTB increased regeneration in a simple HAM conduit so that it is comparable to that achieved with autologous nerve grafting. PTB also proved a rapid and effective method of conferring structural stability to HAM conduits.
34B
DIFFERENTIAL GROWTH FACTOR EXPRESSION IN SUBSETS OF SCHWANN CELLS
Presenter: Manuela Aspalter, MD
Johns Hopkins School of Medicine

Introduction: For the past century, cutaneous nerve has been the preferred graft for nerve reconstruction. However, we have recently shown that grafts of ventral root provide growth factors not found in cutaneous nerve, and are superior to cutaneous nerve in their ability to support motor axon regeneration. In these experiments, we explore growth factor expression by subsets of Schwann cells in cutaneous and muscle nerve to further define the expression patterns of potential nerve grafts.

Methods: Adult rat muscle nerve (MN), cutaneous nerve (CN), dorsal root (DR) and ventral root (VR) were transected surgically and allowed to degenerate for 5, 15, or 30 days. Sub-populations of Schwann cells were denervated using more focal lesions; muscle afferents (MA) by DRG excision, muscle efferents (ME) by ventral rhizotomy, and non-myelinating Schwann cells by application of capsaicin (CN-Cap). Expression of 11 growth factors (NGF, BDNF, NT-3, IGF-1, IGF-2, CNTF, PTN, VEGF, HGF, FGF-2, and GDNF) was evaluated at each time period using competitive, real-time RT-PCR.

Results: The factors that best characterize ventral root, PTN and GDNF, were expressed less (PTN) or minimally (GDNF) in muscle nerve. GDNF was also expressed vigorously in dorsal root, leading to its characterization as a “root” factor, while VEGF and IGF-1 expression was far greater in cutaneous nerve than in dorsal root. BDNF, NGF, and HGF were expressed in cutaneous nerve either more vigorously (HGF, NGF) or more rapidly (BDNF) than in dorsal root.

Conclusions: These data suggest that cutaneous nerve provides an optimal environment for sensory axon regeneration. Muscle nerve, in contrast, cannot provide the same degree of support that was offered by ventral root in our previous experiments. Future efforts will therefore focus on genetic manipulation of readily available cutaneous nerve to maximize its support of regenerating motor axons.

35B
A NEW GENE THERAPY MODEL FOR PERIPHERAL NERVE REGENERATION AND FUNCTIONAL RECOVERY OF RATS USING TRIPLE TRASNFECTED HEK-293 CELLS
Presenter: Jason M. Rogers, BS
Authors: Rogers JM, Dhar S, McConnell MP, Scholz T, Wharton DM, Nguyen TD, Evans GR
University of California – Irvine

Introduction: Our previous in vitro studies have shown the HEK-293 cells can be triple transfected with regulator vector, NGF and HSV-TK to generate a stable cell line (hNGF-EcR-293-TK) which can be regulated to produce NGF upon induction with Ponasterone A and cease NGF production upon ganciclovir treatment. The present study investigated the in vivo NGF regulation and shut-off by these cells and its effect on nerve regeneration and functional recovery.

Materials and Methods: Sprague Dawley rats were used to create a 15 mm sciatic nerve injury with both ends of the nerve sutured into the silastic conduit. Each conduit was filled with 10,000 hNGF-EcR-293-TK cells and divided into various treatment groups and harvested at days 1, 3, 5, 7, 14, 21 and 28 post treatment. Group I had cells in regular media, cells in Group II were induced with Pon A, Group III and IV got induced cells which were further boosted with PonA or treated with GCV, respectively at days 7, 14, 21. Walking track analysis was performed on each rat at all time points for measurement of functional recovery. Media was harvested from all conduits of all the rats at all harvest time points to evaluate NGF expression and bioactivity by ELISA and PC-12 cell differentiation, respectively. Each harvested nerve underwent histomorphometry to determine the nerve fiber density and number of remyelinating axons.

Results: The walking track test demonstrated that the booster group (Group III) rats had highest sciatic functional index (SFI) recovery, whereas rats treated with GCV (Group IV) revealed the lowest SFI index. ELISA and PC-12 bioassay demonstrated that the rats from Group III had highest NGF production (867.24 pg/ml) and PC-12 bioactivity (85.33%), whereas rat in Group IV had the lowest amount of NGF production (96.25 pg/ml) and PC-12 bioactivity (2.66%). Histomorphometrical data analysis is in progress and will be presented.

Conclusion: The study establishes first in vivo gene therapy model of delivering and regulating NGF production using stably transfected HEK-293 cells for peripheral nerve regeneration and recovery of loss of function.
Friday, June 22, 2007

Session 6B
Other
Abstracts 36B – 50B
1:00 – 3:20 pm

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**36B**
**LONG TERM SURVIVAL OF COMPOSITE HEMIFACE/MANDBILE/TONGUE TISSUE ALLOGRAFT PERMITTED BY DONOR SPECIFIC CHIMERISM**

**Presenter:** Yalcin Kulahci, MD  
**Authors:** Bozkurt M, Kulahci Y, Nasir S, Klimczak A, Siemionow M  
Cleveland Clinic

**Introduction:** Extensive head and neck deformities including bone and soft tissue defects are always challenging for reconstructive surgeons. The purpose of this study was to extend application of the face/scalp transplantation model as a new reconstructive option.

**Methods:** A total of 12 composite osseomusculocutaneous hemiface/mandible-tongue transplantations were performed in two experimental groups. Group 1 isografts between Lewis rats (n=6). Group 2 (n=6) composite hemiface/mandible-tongue transplants were performed across MHC barrier between ACI (RT1a) donors and Lewis (RT11) recipients. Hemimandibular bone, masseter muscle, tongue were dissected on the same pedicle of external carotid artery and jugular vein and were transplanted to the donor inguinal region. All allogenic transplant recipients received 16mg/kg/day of CsA monotherapy tapered to 2 mg/kg/ day. All animals were monitored for sign of rejection. Flap angiography and CT scan evaluated allograft viability. Flow cytometry assessed donor-specific chimerism for MHC class I- RT1n antigen. H&E staining revealed bone histology.

**Results:** Isograft controls survived indefinitely. Six allotransplants survived up to 100 days (still under observation). Flap angiography demonstrated intact vascular supply to the bone. No signs of rejection and no flap loss were noted. CT scan and bone histology confirmed viability of bone components of the composite allografts. H&E staining determined the presence of viable bone marrow cells within transplanted mandible. Donor-specific chimerism at day 100 posttransplant was evaluated by presence of donor T-cells (2.7% CD4/RT1n, 1.2% CD8/RT1n) and (B-cells 11.5% CD45RA/RT1n).

**Conclusions:** We have introduced a new model of composite osseomusculocutaneous allograft transplant. Long-term allograft acceptance was accompanied by donor specific chimerism supported by vascularized bone marrow transplant of the mandibular component. This model may serve as a new reconstructive option for coverage of the extensive head and neck deformities involving large bone and soft tissue defects performed in one surgical procedure.

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**37B**
**GENE THERAPY MANIPULATION OF TENDON HEALING: REDUCTION OF ADHESIONS WHILE MAINTAINING TENSILE PROPERTIES**

**Presenter:** Patrick L. Basile, MD  
**Authors:** Basile PL, Dadali T, Jacobson JA, Hasslund S, Schwartz EM, Mitten DJ, O’Keefe RJ, Awad HA, Langstein  
University of Rochester

**Introduction:** Adhesions at the site of tendon repair often limits functional recovery. We investigated the role of growth/differentiation factor-5 (Gdf5), a known determinant in tendon healing, to decrease adhesions but maintain tensile strength in a mouse tendon allograft model compared to a control expressing no known growth factors.

**Methods:** Using recombinant adeno-associated virus (rAAV) mediated gene therapy as a vehicle for delivery, lyophilized mouse FDL (flexor digitorum longus) allografts seeded with rAAV-Gdf5 (n=9) or rAAV-LacZ (n=9) were microsurgically inserted into 3 mm gap defects in the distal FDL of C57BL/6 mice. Tendons were harvested 14 days later, evaluated non-destructively for adhesions and tested biomechanically for repair tensile strength, stiffness, and toughness.

**Results:** All animals survived the surgery and post-op period. Gene delivery via the allograft was verified using bioluminescent and histological markers. Adhesion testing showed that mice receiving the Gdf5 tendon allograft had a statistically significant (p<0.05) decrease in adhesions compared to the LacZ group. Biomechanical testing at this time point showed that there is no statistical difference (p>0.05) in repair strength, stiffness, and toughness between the LacZ and Gdf5 groups.

**Conclusion:** This study demonstrates that application of rAAV-Gdf5 coated tendons decreases adhesions while preserving tensile strength compared to controls, suggesting that Gdf5 can selectively modulate healing at the tendon-tendon interface. These findings may lead to clinical strategies that improve outcomes in tendon healing.
38B
DIABETES AND HIGH GLUCOSE IMPAIR THE ABILITY OF HYPOXIA INDUCIBLE FACTOR-1ALPHA TO INCREASE VEGF TRANSCRIPTION IN RESPONSE TO HYPOXIA
Presenter: Leila Jazayeri, BS, BA
Authors: Jazayeri L, Galiano RD, Ceradini DJ, Chang E, Semenza GP, Longaker MT, Gurtner GC
Stanford University

Introduction: Diabetes is associated with poor outcomes following vascular occlusion likely do to inadequate compensatory microvasculature in response to ischemia. Similarly, poor cutaneous wound healing in diabetics has been linked to impaired neovascularization. Diabetic wounds are deficient in vascular endothelial growth factor (VEGF); an essential mediator of blood vessel growth. Thus we chose to examine whether a selective defect in the hypoxic upregulation of VEGF is present in diabetes and whether this defect can be attributed to hypoxia-inducible factor (HIF), which mediates hypoxia-induced transcription of VEGF.

Methods: Dermal fibroblasts were harvested from type 2 diabetic (n=3) and non-diabetic patients (n=3), incubated in hypoxia and subsequent VEGF secretion measured with ELISA. Diabetic (db/db) vs wt mice were then inflicted with a flap model of soft tissue ischemia and VEGF and HIF 1alpha levels measured in the ischemic flap. Finally, in an in vitro model of hyperglycemia, VEGF and HIF1alpha and 2alpha protein levels and transcriptional activity were examined with ELISA, immunoblots, and two reporter plasmids.

Results: Fibroblasts from diabetic patients did not upregulate VEGF in hypoxia vs controls, which showed a 2.2fold increase (p<0.001). In the murine ischemic flap, VEGF levels in ischemic tissue did not increase in db/db mice vs wt mice (0 vs 2750 pg/mg). Likewise, murine myoblasts in hyperglycemic media produced less VEGF mRNA and protein in hypoxia vs controls. Reporter assays utilizing a full-length VEGF promoter and a construct limited to the consensus HIF-binding element revealed HIF transcriptional activity was impaired by high glucose to a similar extent in both suggesting that interfering factors on the VEGF promoter were not involved.

Conclusion: These data suggest that a selective defect in the hypoxic upregulation of VEGF exists in human and murine diabetics. In vitro, hyperglycemia impairs the hypoxic induction of VEGF at the transcriptional level and impairs HIF transcriptional activity suggesting impaired diabetic upregulation of VEGF occurs via modulation of HIF function.

39B
IN VIVO GENE SILENCING: SIRNA AS A TOPICAL THERAPEUTIC
Presenter: Vishal D. Thanik, MD
New York University

Introduction: The ability to modulate gene expression via topical therapy holds therapeutic potential for conditions ranging from neoplasms to thermal injury and dysfunctional wound healing. Since open wounds represent a substantial biomedical burden and offer a target-rich environment for gene manipulation, we developed a matrix-based method to deliver siRNA topically. MAPK-1 and Lamin A/C candidate genes were selected due to their ubiquitous expression.

Methods: Paired 4mm diameter dorsal wounds were created, stented (12mm O-ring), and covered (occlusive dressing). 24hrs later, liposomal complexed candidate siRNA (20pmol) was incorporated into a cooling 0.4% liquid agarose mixture. This matrix was applied to the wound bed and allowed to gel. On post-injury day 5, siRNA-agarose matrix was removed and the wound re-covered. 48hrs later, a second application of the siRNA-agarose matrix was applied. Wounds (+2mm border) were harvested at days 14 and 21. Tissue was analyzed for target mRNA and protein suppression.

Results: Topical gel-based siRNA locally silenced mapk-1. In situ hybridization demonstrated marked reduction in mapk-1 expression in the wound bed. Real-time RT-PCR of whole wound homogenate (+2mm untreated border) at 14 days demonstrated 49-62% reduction (p<0.003) in mapk-1. Immunohistochemistry revealed marked reduction of mapk-1 in the wound (14/21 days). Similarly, Lamin A/C was effectively suppressed. Gene expression in the surrounding tissue and in the spleen, liver, and lung was unaffected. Western blot analysis of whole wound homogenate (+border) demonstrated marked reduction of mapk-1 and lamin A/C (14/21 days). At day 21, protein-specific suppression persisted.

Conclusion: These results are the first demonstration of effective silencing of ubiquitously expressed genes in an open wound bed with a topically applied siRNA delivery system. Limiting siRNA delivery to the area subjacent to the agarose matrix system allows targeting of genes that cannot be safely or adequately silenced systemically. Similar candidate gene suppression can be achieved by doping the system with alternate siRNAs.
40B
R13-15MER: A SYNTHETIC PEPTIDE FOR SARCOMA THERAPY

Presenter: Frank Jacobsen, PhD
Author: Jacobsen F, Slowicki A, Gevers K, Topal H, Shai Y, Steinau HU, Steinstraesser L
BG University Hospital Bergmannsheil, Ruhr University Bochum

Introduction: Sarcomas are heterogenous, mesenchymal tumors of high malignancy. Additionally, sarcomas frequently showed drug resistance for chemotherapeutics, which demands alternative therapeutical options, as surgical resection is often limited. This study demonstrates the efficiency of an antimicrobial peptide derivative in sarcoma therapy.

Methods: The peptide was analyzed in vitro against primary sarcoma cells and the fibrosarcoma cell line HT1080 using MTT-Assay and BrdU-Assay. Afterwards, athymic mice (n=20) subcutaneously received 106 HT1080 cells within a Matrigel matrix on its back. After 7 days the mice were randomized into treatment (R13-15mer, n=10) and control (PBS, n=10) group. Peptide (1 mg/kg) and PBS were intratumorally injected thrice a week for a three weeks follow-up. Finally the mice were euthanized and the tumor was dissected for histological expertise.

Results: The R13-15mer showed cytotoxicity against both the primary sarcoma cells and the HT1080 cell line within the in vitro assays. The mice model demonstrated a reduced tumor growth and after tumor dissection, a significantly reduced mean tumor weight of about 45% within the treatment compared to the PBS control group. The histological analysis demonstrated reduced amounts of solid tumor tissue and blood vessel support within the peptide treated tumors.

Conclusion: The use of R13-15mer as an anti sarcoma therapy was successful. However, a complete remission was observed in no case. Further studies are necessary to increase the efficiency of this peptide and to investigate its potential for systemic application.

41B
ADIPOGENIC DIFFERENTIATION OF ADIPOSE-DERIVED STEM CELLS IN A NOVEL HOLLOW FIBER-BASED BIOREACTOR

Presenter: Kacey G. Marra, PhD
Authors: Marra KG, Rubin JP, Brayfield CA, Baun M, Gerlach JC
University of Pittsburgh

Introduction: Adipose-derived stem cells (ASCs) provide an abundant source of progenitor cells that can be expanded in culture and used in cell-based therapies to correct contour defects. In order to fully assess the potential for primary adult human ASCs to develop natural adipose tissue architecture, we cultured ASCs in a hollow fiber-based bioreactor.

Materials: ASCs were isolated from abdominal subcutaneous adipose tissue harvested from a 51 year old female. 8x10^7 ASCs were inoculated into a hollow fiber-based four-compartment bioreactor with integral oxygenation and counter-current mass exchange with low gradients via interwoven artificial capillaries developed by our group. 3x10^3 ASCs were kept for 2D controls in flasks in a CO2 incubator. Cells were cultured for an expansion period of 28 d, then differentiated for 21 d with adipogenic media. On day 50, the tissue/fiber samples were removed from the bioreactor. Samples were stained with Oil Red O to analyze lipid inclusion and stained with H&E and Masson’s Trichrome to determine matrix formation.

Results: Within the first wk, ASC glucose consumption and lactate production initially increased, then decreased and plateaued. During the adipogenic differentiation period, glucose consumption and lactate production steadily increased possibly due to differentiating cells storing glucose similar to adipocytes. At day 50, adipose tissue formation was seen macroscopically. Oil Red O staining on frozen sections showed that all vacuoles within the aggregates corresponded to ASCs that had differentiated into adipocytes. Results in 2D flasks showed lipid inclusion in some, but not all cells, and did not result in a similar architecture of larger lipids surrounded by ECM.

Conclusions: The 4-compartment hollow fiber-based bioreactor was successful in providing the proper environment for ASC differentiation in vitro. Compared to 2D controls, bioreactor samples resulted in significantly improved ECM formation and full lipid inclusion similar to natural adipose tissue architecture. This represents a useful model for studying the development of cell-based therapies.
42B
THE SUBCUTIS IN HEALTHY INDIVIDUALS IS ISCHEMIC DURING SITTING WHICH IMPLICATES MULTI-LEVEL DAMAGE IN PRESSURE ULCER DEVELOPMENT.
Presenter: Johan Thorfinn, MD, PhD
Authors: Thorfinn J, Sjoberg F, Lidman D
Dept of Biomedicine and Surgery (Linkoping University, Faculty of Health Sciences)

Introduction: Pressure ulcers in the tissues overlying the ischial tuberosities are common in patients confined to wheelchairs. It is generally assumed that the degree of ulcer development is related to the level of sitting pressure, but the scientific evidence is not well substantiated. Moreover, there is a debate whether early pressure ulcer development involves superficial or deep tissues, or both.

Methods: Healthy controls (n=10) were alternating between sitting on a wheel chair cushion (WCC), a hard surface (HS), and unloaded rest. Partial tissue oxygen pressure (tpO₂) was measured in the subcutaneous tissue overlying the ischial tuberosities using an oxygen sensitive microelectrode (Integra Lifesciences, USA). Concentrations of tissue glucose, lactate, pyruvate and glycerol were quantified with microdialysis (CMA Microdialysis, Sweden) on the contralateral side. Sitting pressures were measured using a pressure sensitive mat (Tekscan, USA).

Results: Concentrations of glucose and tpO₂ decreased significantly both when sitting on WCC and on a HS compared to pre- and postloading values. Sitting on a HS resulted in significantly larger reduction in tpO₂, significantly higher sitting pressures, and tendency to a higher lactate/pyruvate ratio.

Conclusions: This study is to our knowledge the first evidence that ischemia develops early in the subcutaneous adipose tissue in the gluteal area in healthy individuals when sitting. Severe long-standing tissue ischemia will cause tissue necrosis and wound formation. The level of the ischemic insult seems to be dependent on the level of sitting pressure since sitting on a WCC resulted in larger reduction of tpO₂. The study stresses the importance of using sitting pressure as a method of identifying individuals prone to develop pressure ulcers. Moreover, it implicates that early pressure ulcer development in the gluteal area engages the subcutaneous tissues, supporting the theory that gluteal pressure ulcers evolve skin deep.

43B
DIFFERENTIATIVE AND PROLIFERATIVE CAPACITY OF ADIPOSE AND DURAL STEM CELLS CULTURED ON PLAGA MICROSHERES
Presenter: Caren Petrie Aronin, MD
Authors: Tholpady SS, Tholpady A, Petrie CP, Sadik KW, Ogle RA, Katz AJ, Botchwey E, Ogle RC
University of Virginia

Introduction: Efforts directed at creating regenerative templates for tissue repair have centered on a fairly straightforward paradigm: a defined biomaterial seeded with cells bathed in specified growth factors. In this manner, multiple tissue types have been modeled in vitro and studied in vivo. In this study, the interactions between adipose stem cells (ASCs), dural stem cells (DSCs) and poly lactic co-glycolic acid (PLAGA) constructs are analyzed for the ability to create and support a bony architecture and phenotype.

Methods: Multipotentiality of ASCs and DSCs was proven by differentiating these cell types into bone and nerve. PLAGA microspheres were scintered into semi-rigid matrices by a previously described method. These matrices were cultured with ASCs and DSCs in a bony differentiative media. Over the course of a three week period, these constructs were analyzed for bony genotype generation by immunohistochemistry and RT-PCR.

Results: Both ASCs and DSCs were able to differentiate into osteoblasts when placed in defined media. These media were found to be differen, however, as the steroid normally present in osteoblastic differentiative media caused the DSCs to gain a neural phenotype. Thus the steroid was omitted. It was found that while both ASCs and DSCs were capable of osteoblastic differentiation, DSCs were more likely to adhere to the PLAGA and form a dense layer of osteoblasts and osteoblastic matrix.

Conclusion: It was determined that DSCs have properties that are more amenable to large scale tissue engineering of bone on PLAGA. They were shown to have an increased expression of bony markers both at the RNA and protein levels. Because of these enhanced ability to adhere to PLAGA, they were able to create a more dense osteoblastic layer that would contribute to bone formation in vivo.
**44B**  
**EPIDENETIC ABNORMALITIES OF INFANTILE HEMANGIOMAS**  
**Presenter:** Brent E. Schultz, BS  
**Authors:** Schultz BE, Persing J, Halaban R, Zide B, Waner M, Narayan D  
Yale Department of Surgery  

**Introduction:** Insulin Like Growth Factor 2 (IGF2), a genetically imprinted tumorigenic growth factor, is strongly implicated in the pathogenesis of infantile hemangiomas. Given the substantial documentation that methylation abnormalities within the IGF2 locus is related to the overproduction of IGF2 in many distinct tumor types, an investigation of the methylation status of this region may explain pathologic IGF2 overproduction in hemangiomas.

**Methods:** Tissue from 14 hemangiomas—5 proliferative, 5 involuting, and 4 involuted—with matched peripheral blood controls were subjected to methylation sensitive enzymatic digest and genomic Southern hybridization. Both the H19 differentially methylated region as well as the neighboring imprinting control region LIT1, were probed. Methylation status was expressed as a methylation index (MI): a quotient of the brightness intensity of the methylated band (uncut by methylation sensitive restriction enzymes) divided by the brightness intensity of the unmethylated band (cut.) Normal methylation is .5 with a standard deviation of .07.

**Results:** All control blood samples demonstrated normal methylation patterns at both H19 and LIT1. Moreover, all tumor samples were normally methylated at LIT1. However, in all samples tested, the H19 imprinting control region was severely hypomethylated with MI’s ranging from 19% to 35% (P = .00001.) Plotting the methylation index against the age of the lesion removed, indicates that Infantile Hemangiomas continue to demethylate at the H19 imprinting control region over time.

**Conclusions:** Hypomethylation of the H19 locus is the first consistent genetic phenomenon to be associated with non-familial, non-syndromic, Infantile Hemangiomas. Normal blood and Lit1 methylation suggest that this hypomethylation is both tissue and gene specific. Given our current understanding of IGF2 regulation, these findings are consistent with IGF2 down regulation, and may go to explain this phenomenon in involuting hemangiomas.

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**45B**  
**COMPARISON OF CHIMERISM LEVEL AND FLAP SURVIVAL AFTER PRESENTISIZED BONE MARROW ALLOGRAFTS AT 24 HOUR AND 72 HOUR**  
**Presenter:** Łukasz Krokowicz, MD  
**Authors:** Bozkurt M, Nasir S, Luszczek W, Klimeczak A, Siemionow M, Krokowicz L  
Cleveland Clinic  

**Introduction:** Composite tissue allografts (CTAs) offer a great potential for functional and cosmetic restoration. However the main obstacle precluding routine use of CTAs is the need for life-long immunosuppressive therapy. Bone marrow transplantation is suggested to provide donor–specific immune-tolerance induction through establishment of mixed lymphoid chimerism. We proposed to evaluate effect of donor sensitisation on the immune response to vascularized skin allografts transplants in allograft recipients.

**Methods:** Twenty transplantations were performed in 4 experimental groups of 5 animals each. Allograft transplants were performed between presentisized ACI donors and Lewis recipients. Group I and II groups were the control groups which vascularized skin allografts (VSA) were transplanted and no immunosuppressive treatment was given after the transplantation. In all groups the ACI (RT 1a) rats were presentisized with Lewis (RT1 I) bone marrow and these donors did not received any immunosuppressive therapy. At 24 hour and 72 hour after sensitisation vascularized skin allografts (VSA) were transplanted to Lewis (RT1 I) rat in group III and IV which received a combination of αβ TCR and cyclosporine (CSA) treatment for a week. Assessment included flap viability, flow cytometry for chimerism and immunohistochemistry.

**Results:** In the control groups (group I,II) vascularized skin allografts (VSA) were acutely rejected within 5 to 9 days post transplant. Donor specific chimerism at day 7, 21, 35 and 63 were evaluated by presence of T-cells (ACI/CD 4 and ACI/CD 8) and B cells( ACI/CD 45 and ACI / CD 11). Group III and IV is still under observation and preliminary results indicate that chimerism levels are higher in group IV where transplant was sensitised (72 hour after marrow transplantation).

**Conclusions:** Donor sensitisation with recipient bone marrow modifies the recipient’s responsiveness to vascularized skin allografts under short-term αβ TCR and cyclosporine (CSA) therapy.
46B
THE ISCHEMIC RESPONSE OF BONE MARROW PROGENITORS IS ALTERED IN TYPE II DIABETES
Presenter: Jacquelyn Carr, BA
New York University Medical Center

Introduction: Neovascularization occurs via endothelial cell sprouting and endothelial progenitor cell (EPC) differentiation. Ischemic complications can result from impairments in either process. We have previously demonstrated that circulating diabetic (db) EPCs are impaired in number and function, yet little is known about how ischemia effects the db bone marrow (BM) niche.

Methods: Lineage depleted BM cells from db and wild type (wt) mice were studied for proliferation, adhesion, and growth factor production. EPC mediators (VEGF, SDF1?, MMP-9 and SCF) were examined under normoxic (21% O2) and hypoxic (1% O2) conditions. A flap model was used to study the in vivo effects of ischemia on BM EPC (lin-flk+sca+ckit+) mobilization.

Results: Db EPC adhesion to collagen and vitronectin was significantly impaired in hypoxia (8±2 and 16±3 vs 19±6 and 38±9 per HPF, <0.05). Db EPCs exhibited an impaired proliferation index at baseline (0.13±0.05 vs 0.29±0.03, <0.01), but a comparable 3.3-fold (p<0.001) increase in proliferation at 7 days post-ischemia. BM FACS analysis revealed similar trends in proliferation at day 7 (5-fold increase); however, at day 14, wt EPC levels returned to baseline while db EPCs remained elevated (6±1% lin-sca+ vs 39±2%, p<0.01). MMP-9 appeared to originate in the BM, as db BM MMP-9 production in vitro was higher in both normoxia and hypoxia (p<0.01). A concomitant magnified response in circulating SCF was observed at day 7 (27±12 vs 12±4.5 pg/mL, p<0.05), which may have resulted from a greater ischemic insult at these time points (151±42 vs 306±55 Doppler flow units at day 2; p<0.05).

Conclusion: This is the first study to conclude that db EPC dysfunction may arise from their inability to mobilize from the hypoxic BM. Despite adequate EPC proliferation, as well as elevated MMP-9 and s-kit, db EPCs fail to respond to ischemia-induced mobilization signals. Ongoing studies investigate this mechanism with hopes of designing targeted therapies to improve the diabetic response to injury.

47B
CRYOPRESERVATION OF FAT GRAFTS HARVESTED WITH THE COLEMAN TECHNIQUE: A COMPREHENSIVE EVALUATION
Presenter: Lee L. Pu, MD,PhD
Authors: Pu LL, Cui X, Coleman SR, Ferguson RE, Vasconez HC
University of Kentucky

Introduction: Banking of fat grafts for future applications may have significant impact on autologous fat transplantation in plastic surgery. However, the viability of fat grafts harvested with a standard technique after cryopreservation remains unknown. This study is conducted to evaluate the viability of cryopreserved fat grafts harvested with the Coleman technique.

Methods: Eight adult white females were enrolled in this self-controlled study. In each patient, 10 cc of fat grafts were harvested with the Coleman technique by a single surgeon (SRC) from the lower abdomen according to his well described method. In Group 1(N=8), 5 cc of fresh fat grafts were mixed with cryoprotective agents (0.5 M dimethyl sulfoxide and 0.2 M trehalose) and then underwent cryopreservation with controlled slow cooling and fast rewarming. In Group 2 (N=8), 5 cc of fresh fat grafts from the same patient without cryopreservation were served as a control. The fat graft samples from both groups were analyzed with trypan blue vital staining for viable adipocyte counts, glycerol-3-phophatase dehydrogenase (G3PDH) assay for intracellular enzyme activity of fat grafts, and routine histology.

Results: There was a decrease of viable adipocyte counts in Group 1 compared with Group 2 (3.46 ± 0.91 vs. 4.12 ± 1.11 x 1,000,000 Cells/ml, Mean ± SD) However, the difference between both groups was not statistically significant (p=0.22). There was a significant decrease of the G3PGH activity in Group 1 compared with Group 2 (0.47 ± 0.09 vs. 0.66 ± 0.09 u/ml, p<0.001) Histologically, the normal structure of fragmented fatty tissues was found primarily in both groups.

Conclusions: Our results indicate that cryopreserved fat grafts harvested with the Coleman technique have a normal histology with near the same number of viable adipocytes as compared with the fresh fat grafts. However, cryopreserved fat grafts appear to have a less optimal level of adipocyte specific enzyme activity compared with the fresh ones and thus may not survive well after they are transplanted without being optimized.
**48B**

**IMMUNE REACTION AFTER ADENOVIRAL GENE DELIVERY INTO SKIN**

**Presenter:** Michael Sorkin, BS  
**Authors:** Jacobsen F, Sorkin M, Gevers K, Rittig A, Steinau HU, Steinstraesser L  
**BG University Hospital Bergmannsheil, Ruhr University Bochum**

**Introduction:** The transient cutaneous gene transfer will be a common alternative to treat several skin diseases in future. The adenoviral vector system showed highest effectiveness including its immunogenicity as the only detriment, which potentially make this vector system critical for reapplication. The aim of this study was to quantify the local and systemic immune reaction after a reapplication of adenoviral vectors.

**Methods:** Hairless, immuno-competent mice were randomized into three different groups. Adenoviral vectors containing the GFP-transgene were intradermally injected at concentrations of $10^8$, $10^9$ or $10^{10}$ IU (infective units). Afterwards, the transgene expression was quantified every second day using a Kodak imaging system (4000MM). The same amount of viral vectors was applied on one hand into the same pre-treated areas on the other hand additive into an untreated skin area of the same mouse at day 14 and 28. Additionally blood was taken at day 0, 14 and 28 to analyse serum concentration of adenovirus specific antibodies.

**Results:** The GFP expression strictly depended on the corresponding viral load. Only for the amount of $10^8$ and $10^9$ IU a transgene expression was above background level within the first 12 days whereas the highest level was seen at day 5. After the second and third application transgene expression was only measured within the first two days and decreased to background level until day 5. The corresponding antibody concentration continuously increased during the treatment.

**Conclusion:** The use of adenoviral vectors for transient cutaneous gene delivery induced local and systemic immune reaction, which correlated with the applied viral load. The immune response drastically decreased the efficiency of the vector system after reapplication, which disqualifies the use of adenoviral vectors for gene transfer repetition.

**49B**

**THE EFFECT OF IN-UTERO CLEFT PALATE REPAIR ON LEVATOR VELI PALATINI MUSCLE STRUCTURE AND FUNCTION**

**Presenter:** Paul S. Cederna, MD  
**Authors:** Cederna PS, Weinzweig J, Buchman SR, Yu D, Panter KE, Faulkner JA, Larkin LM  
**University of Michigan Health System**

**Introduction:** We hypothesize that inherent difference in the levator veli palatini muscle of clefted palates prior to surgical repair plays a critical role in the persistent rate of velopharyngeal insufficiency (VPI) after cleft palate repair. Specifically, we posit that there is a temporal relationship between the inherent structural and physiologic differences in the levator veli palatini (LVP) muscle of cleft palates (CP) to a maladaptive response over time. The purpose of this study was to determine if earlier, in utero repair (IUR), of the CP would have a beneficial effect on the LVP muscles and potentially improve palatal function after repair. We theorized that performing in utero repair would provide the optimal conditions for healing such that the LVP muscles would closely resemble normal palatal (NP) musculature.

**Methods:** The LVP muscle from 12 Spanish goats were evaluated:  
1) CP goats, undergoing IUR, evaluated at 2 months of age (n=3);  
2) CP goats 2 months of age, unrepaired clefts (URC) (n=4); and  
3) NP goats 2 months of age (n=5). Pregnant goats were gavaged with an abasine during palatal shelf closure (38 days gestation) to induce the CP. CP’s were repaired at 85 days gestation utilizing a Von Langenbeck approach. Goats were then harvested at 2 months of age and histomorphometric analysis was performed. Western blot analysis was performed to determine myosin heavy chain (MHC) content.

**Results:** LVP muscles from IUR cleft palates (2450+179 µm²) demonstrated similar cross sectional areas (CSA) to NP (2349+151 µm²), and were smaller than the CSA for CP’s (2981+182 µm²). 72% of the muscle fibers in the IUR group were slow, oxidative, fatigue resistant (Type I) muscle fibers as compared to 79.5% of the NP’s. In contrast, the URC LVP muscles demonstrated only 56.4% Type I muscle fibers, which was significantly lower than that seen in either the normal or IUR palates. Western blot analysis confirmed a mixed fast/slow MHC content in the URC group and predominantly slows MHC content in the normal and IUR palatal groups.

**Conclusion:** Clear anatomic and physiologic differences exist between the cleft and normal palates that may explain the variance in cleft palate repair outcomes. IUR of the cleft palate results in a transformation of the LVP muscle to that which closely resembles normal palatal LVP muscle; a concomitant improvement in palatal function could well result in a reduced incidence of VPI following repair.
50B
CYCLIC STRAIN INDUCES
REDISTRIBUTION OF INTEGRINS
ALPHA3 BETA1 AND THE TYROSINE
PHOSPHORYLATION OF FOCAL
ADHESION KINASE (FAK) IN DERMAL
FIBROBLASTS

Presenter: Bauer Sumpio, MD, PhD
Authors: Wen H, Blume PA, Sumpio BE
Yale University School of Medicine

Negative pressure wound therapy is a unique system that helps promote wound healing. However, the mechanisms by which fibroblasts sense and respond to physical forces remain to be elucidated.

Cultured human dermal fibroblasts grown on collagen 1 matrix were exposed to 10% strain at a frequency of 10 cycles/min. Cells were stained with specific antibody to integrins beta1, alpha1, alpha2, and alpha3. The activation of FAK was assessed by immunoblotting with anti-total FAK antibody and anti-phospho-FAK (Tyr576/577) antibody.

Results: Under static conditions, the cell axis is random. After 2hr stretch, cells rotate to perpendicular to stretch. The distribution of beta1 under static condition is diffuse. After exposure to 30 min stretch, beta1 is more clustered and reorganized around the nucleus. alpha3 under static conditions also shows a diffuse pattern. After 30 min stretch, alpha3 is concentrated and reorganized around nucleus. alpha1 and alpha2 staining is much weaker and did not change with stretch.

Conclusions: 10% 10cpm cyclic stretch can cause clustering of integrin alpha3, beta1 and the phosphorylation of FAK. This suggests that the integrin-FAK pathway may be a critical signaling sensor in fibroblasts exposed to mechanical forces.
Saturday, June 23, 2007

Session 8B
Microsurgery
Abstracts 51B – 56B
8:00 – 8:50 am

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**51B**

**SPIDER SILK FIBRES IN ARTIFICIAL NERVE CONSTRUCTS PROMOTE PERIPHERAL NERVE REGENERATION**

**Presenter:** Peter M. Vogt, MD, PhD  
**Authors:** Allmeling C, Jokuszies A, Reimers K, Kall S, Choi CY, Brandes G, Kasper C, Vogt PM

**Medical School Hannover**

**Introduction:** Replacement of lost body structures with bioengineered grafts are intensively studied alternatives to current surgery. Bridging neural defects by autologous material is limited, while artificial neural conduits never achieved the regenerative capacity of autologous grafts. In our study, we found that spider silk, a new material in nerve tissue engineering, incorporated into isogenic veins showed an excellent performance in neuronal regeneration.

**Methods:** 20 mm sciatic nerve defects in rats were replaced. We compared four experimental groups: isogenic nerve grafts, vein grafts with spider silk fibres, vein grafts with spider silk fibres supplemented with Schwann cells and vein grafts with spider silk fibres, Schwann cells and matrigel (n=5). A control was transplanted with veins and matrigel (n=5). Nerve conductance was demonstrated and the gastrocnemius muscles were harvested bilaterally. The grafts were explanted and analysed histologically, immunofluoroscopically and in electron microscopy.

**Results:** Nerve regeneration could be achieved with all constructs except in the control group. Best results were obtained with nerve constructs consisting of veins and cell-free spider silk and constructs consisting of veins, spider silk, matrigel and Schwann cells. The muscle-weight ratio of the ipsilateral to the contralateral M. gastrocnemius was 66.6% ± 11.44 for vein grafts with spider silk fibres, Schwann cells and matrigel compared to 78.2 ± 6.64 isogenetic grafts. The regenerated nerves stained for S-100 and Neurofilament. Axonal regrowth and myelin sheaths could be demonstrated in electron microscopy.

**Conclusion:** Our results describe the suitability of veins filled with spider silk as artificial nerve grafts. Nerve regeneration equivalent to isograft controls could be achieved. Interestingly, in contrast to literature, we found that bridging an extensive nerve gap by cell-free constructs with spider silk was highly efficient. This approach holds the promise to replace the current methods of peripheral nerve repair.

**52B**

**EX-VIVO GENE TRANSDUCTION OF MICROVASCULAR FREE FLAPS WITH ANTIMICROBIAL PEPTIDES TO TREAT CHRONIC INFECTIONS**

**Presenter:** Shadi Ghali, MD  
**Authors:** Ghali S, Bhatt KA, Dempsey MP, Hamou C, Jones DM, Chang E, Singh S, Shi Y, Gurtner, GC

**Stanford University**

**Introduction:** Recalcitrant infections often require microsurgical free tissue transfer to fill soft tissue and osseous defects. Despite the transfer of vascularized tissue, there is still a 30-40% failure rate due to infection. Antimicrobial peptides are a ubiquitous group of natural molecules with minimal pathogenic resistance. This study examines the utility of using free flaps in a therapeutic as well as reconstructive capacity to deliver the human antimicrobial peptide LL37.

**Methods:** Superficial inferior epigastric (SIE) fasciocutaneous flaps were raised in adult Fischer rats and transduced ex-vivo with adenovirus-LacZ reporter gene (4.6x10⁹ PFU) or adenovirus-LL37 (4.4x10⁹ PFU) +/- vascular endothelial growth factor (VEGF) to improve transduction efficiency. A bioluminescent S.aureus catheter infection model was established to determine the biological efficacy of this transduction method. Bioluminescent photon emissions were monitored non-invasively using an IVIS camera and final bacterial counts were analyzed 7 days following an initial inoculation of ~4x10⁸cfu/m bioluminescent S.aureus.

**Results:** Maximal transgene expression following transduction with recombinant adenovirus was demonstrated by ELISA at day 7. The co-administration of VEGF with recombinant adenovirus-LL37 resulted in a 50% increase in flap transduction efficiency and a 100% increase in peri-flap transgene expression. Flaps transduced with adenovirus-LL37 demonstrated significantly lower bacterial counts compared to controls at day 7 (~2.5x10⁸ cfu/ml vs ~3.4x10⁷ cfu/ml). Flaps with adeno-LL37 + VEGF demonstrated a further significant improvement in bacterial clearance compared to controls (~1.1x10⁴ cfu/ml vs ~3.4x10⁷ cfu/ml).

**Conclusions:** We have demonstrated successful transduction of the reporter gene, LacZ as well as the therapeutic gene product, LL37 using ex-vivo gene delivery methods. The gene product was expressed maximally at day 7 and was concentrated in the flap and peri-flap tissues, where recalcitrant infection commonly occurs. Transduction efficiency was improved with the addition of VEGF and biological efficacy was demonstrated in a rat infection model. We believe that the ex-vivo gene transduction of microvascular free flaps with therapeutic genes is feasible, efficacious and safe and has enormous potential in the field of reconstructive plastic surgery.
53B
LOW DOSE RADIATION IMPROVES PERfusion AND VASCULARITY IN AN ISCHEMIC FLAP MODEL
Presenter: Oren Z. Lerman, MD
New York University School of Medicine

Purpose: Increasing evidence suggests that low dose radiation (XRT) can be pro-angiogenic. We have previously demonstrated that XRT stimulates HIF1alpha expression and the CXC chemokine SDF1 in cultured endothelial cells. We hypothesize that low dose XRT augments neovascularization in ischemic tissue by stimulating mobilization of endothelial progenitor cells (EPCs) through HIF1alpha induced expression of SDF1.

Methods: A murine dorsal ischemic flap was created and locally irradiated with a single dose of either 2 or 5 Gy. Flap blood flow was measured by laser doppler at the days 0, 2, 7 and 14 and subsequently harvested. Vascularity was assessed by immunohistochemical CD31 staining. Concurrent EPC mobilization was measured by identification of Lin-/Sca1+/VEGFR2+ cells in peripheral blood by flow cytometry. Identical experiments were performed in a subset of transgenic mice expressing LacZ under the control of an endothelial-specific promoter (Tie2/LacZ) to further examine vascularity.

Results: Blood flow as measured by laser doppler to the ischemic flap increased 60% at 14 days after 2Gy XRT (p<0.05) and 25% and 100% at 7 and 14 days after 5Gy XRT (p<0.05) compared to ischemia alone. Whole mounts of Tie2/LacZ flaps confirmed increased vascular patterning on a gross level. Further histologic evaluation of vascular density revealed a 20% and 60% increase 14 days after 2 and 5 Gy XRT, respectively (p<0.05), thus suggesting neovascularization rather than vasodilatory effects. Flow cytometry demonstrated increased circulating EPCs (57% and 73%) 7 days after 2 and 5 Gy XRT (P<0.05).

Conclusion: Low dose XRT augments neovascularization in an ischemic flap. Circulating EPCs are significantly elevated in mice receiving local XRT compared to controls. These results demonstrate that a single low dose of local XRT induces an angiogenic response in ischemic tissue by mobilization of endothelial progenitor cells.

54B
IMPAIRED NEOVASCULARIZATION AFTER HIGH DOSE RADIATION INJURY – THE ROLE OF EPCS
Presenter: Christopher C. Chang, BA
New York University Medical Center

Introduction: High dose ionizing radiation (XRT) can lead to irreversible damage and poor tissue repair capacity. Impaired angiogenesis after radiation injury has been well documented. Endothelial repair and vasculogenesis through endothelial progenitor cell (EPC) recruitment has not been evaluated despite the fact that EPCs can be responsible for up to 35% of neovascularization. We hypothesize that impaired vasculogenesis plays a central role in the pathophysiologic process that leads to long term complication of XRT.

Methods: Human umbilical vein endothelial cells were exposed to 20Gy XRT and 1% hypoxia. HIF1 activity was measured by Western blot. SDF1 and VEGF expression were measured by quantitative real time RT-PCR. Neovascularization and EPC mobilization were analyzed in a mouse ischemic skin flap model locally exposed to 20Gy XRT. Blood flow was measured by laser Doppler. Vascularity was assessed by CD31 staining. EPC mobilization was measured by identification of Flk1+/Sca1+/Lin- cells in peripheral blood on flow cytometry.

Results: HIF1 protein upregulation in hypoxia was mildly impaired after XRT. SDF1 mRNA levels although initially elevated (11.89 vs 5.99 p<.05) dropped sharply after 48 hrs (4.28 vs 8.55 p<.05). VEGF expression was elevated (23.75 vs 5.2 p<.05). Vascular perfusion on laser Doppler decreased by 50% after high dose XRT on day 7 (p<.05). EPC levels in the peripheral blood were increased 2.5 fold (.35% vs .86% p<.05) after 20Gy XRT.

Conclusion: EPC mobilization after XRT is significantly elevated. Neovascularization after 20Gy, however, remains impaired in our murine flap model. VEGF and SDF1 demonstrated a robust initial upregulation. SDF1 production decreased sharply after 48 hours. We conclude that EPCs are mobilized by an initial upsurge in vasculogenic stimuli. Once mobilized they are unable to migrate to the damaged tissue due to a falloff in the chemokine SDF1 responsible for EPC homing. The capacity to recruit uninjured EPCs from the periphery to repair or replace injured vessels would be a powerful therapeutic tool for the treatment of long term sequelae of radiation injury.
55B
VAC DRESSING CHANGE PAIN ASSESSMENT USING TOPICAL LIDOCAINE VS. PLACEBO: A DOUBLE BLIND, PROSPECTIVE, RANDOMIZED STUDY
Presenter: Jayant P. Agarwal, MD
Authors: Agarwal JP, Dorafshar AH, Lohman RF, Franczyk M, Gottlieb LJ
University of Chicago Hospitals

Background: Analgesic drug therapy is often necessary to facilitate VAC dressing changes, especially during early stages of treatment. Opioids are commonly used, but have side effects. Local anesthetics may avoid many of the problems associated with opioids.

Methods: A prospective, double blind, randomized, placebo controlled study was performed with in-patients (N=65) undergoing multiple VAC dressing changes at a single institution between 12/03 and 6/05. Patients were randomized to receive either 0.2% lidocaine or 0.9% saline. Solutions were administered through the VAC suction tubing into the foam dressing 30 minutes prior to each dressing change. All patients received morphine sulfate ad lib and rated their pain score according to the 0-10 numeric pain distress scale method. Differences in pain scores, the amount of morphine utilized, heart rate, blood pressure, and lidocaine dose between groups were tested.

Results: The mean age of the patients was 54 (range 23 to 81). There were 20 males and 45 females. Patients receiving lidocaine experienced less pain than did control patients during and immediately after the first dressing change (p<.05). Thirty minutes after the dressing change, pain scores returned to baseline levels and did not differ between groups. Pain scores did not differ significantly between groups after the first dressing change.

Opioid use was the same during the first dressing change for both groups. However, opioid use was greater among patients who received lidocaine in the first half hour after the dressing change. Other measures of pain and anxiety, namely blood pressure and heart rate were similar in both groups.

Conclusions: During the initial VAC dressing change, 0.2% lidocaine administered via the suction tubing led to a reduction in pain experienced by patients in our study. The impact of the lidocaine may be short lived as patients in this group requested more opiates in the period after the dressing change.

56B
NOTCH SIGNALING IS REQUIRED FOR ADULT DERIVED STEM CELL PROLIFERATION AND OSTEOGENIC DIFFERENTIATION
Presenter: Damon S. Cooney, MD, PhD
Authors: Cooney DS, Chambers C, Neumeister M
Southern Illinois University School of Medicine

Introduction: Tissue engineering holds great promise for reconstructive/regenerative medicine. Adipose derived stem cells (ADSC) have been touted as an easily obtainable population to use in such projects; however, little is known regarding the molecular mechanisms that regulate ADSC physiology. The present study investigated how inhibition of endogenous Notch signals impacted ADSC proliferation and osteogenic differentiation.

Methods: ADSC isolated from the inguinal fat pads of male Lewis rats were plated at 2 different densities (1,300 cells/cm2 or 5,200 cells/cm2) and treated with either vehicle or a Notch inhibitor (DAPT), which prevents a critical proteolytic step during Notch activation. For the proliferation studies, cells were grown for 5 days in growth media containing either 0.1% DMSO (vehicle) or 1microM DAPT (Sigma). Proliferation was assessed by the MTT assay; cell numbers were extrapolated from a MTT standard curve. For the differentiation experiments, ADSCs were grown for 3 days in osteoinduction media only then on the 4th day, either DMSO (0.1%) or DAPT (1microM) was added. Cells were incubated for an additional 4 days; matrix deposition was assessed by Alizarin Red (AR) staining and quantitation.

Results: DAPT treatment of rat ADSC resulted in a significant reduction in cell proliferation at both low and high cell plating densities. This effect was confirmed by manual cell counts as well. DAPT treatment of osteoinduced ADSC resulted in a significant reduction in extracellular matrix deposition in ADSC plated at low density. This effect was not seen at the higher cell density plating. However, similar amounts of AR staining were found in low density DAPT treated ADSC, high density DMSO- and DAPT-treated ADSCs.

Conclusions: This study demonstrates the importance of endogenous Notch signals on ADSC physiology, both at the level of proliferation and differentiation. Notch inhibition effectively reduced ADSC proliferation and negatively regulated extracellular matrix production by osteoinduced ADSC. Furthermore, ADSC matrix production may be compromised by high cell densities.
Saturday, June 23, 2007

Session 9B
Fetal Surgery/Wound Healing
Abstracts 57B – 65B
9:30 – 10:50 am

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57B

CYTOKINE MODULATION OF THE ANGIOGENIC TRANSCRIPTION FACTOR HOXD3 IN HUMAN MICROVASCULAR ENDOTHELIAL CELLS

Presenter: Peter J. Ro, BS
Authors: Ro P, Mace KA, Dosanjh A, Yu DH, Cheung R, Boudreau N, Young DM, Hansen SL

University of California, San Francisco

Introduction: Wound healing is a complex multistep process and is impaired in diabetics. In diabetics, there is dysregulation of cytokine expression. Expression of the transcription factor Hoxd3 is decreased in diabetic wounds of mice, and gene transfer of HOXD3 improves diabetic wound healing. In this study, we evaluated cytokines that are important during the inflammatory and proliferative/granulation phases of wound healing to determine whether they are upstream regulators of HOXD3 expression.

Methods: We evaluated HOXD3 mRNA expression in human microvascular endothelial cells (HMEC) using quantitative PCR. Total RNA was isolated from HMECs treated with transforming growth factor-beta (TGF-b) and tumor necrosis factor-alpha (TNF-a) after 4 hours. RNA was also isolated from HMECs treated with insulin-like growth factor-1 (IGF-1) and basic fibroblast growth factor (bFGF) after 4 and 8 hours.

Results: After 4 hours of treatment with cytokines TGF-b and TNF-a, HOXD3 mRNA levels were decreased by 44.0% and 50.0%, respectively. After 4 hours of treatment with IGF-1 and bFGF, there were minimal changes in HOXD3 mRNA levels. However, after 8 hours of IGF-1 and bFGF treatment, HOXD3 mRNA levels were increased by 83.3% and 21.2%, respectively.

Conclusions: Sequential cytokine expression after wounding plays an important role in the wound healing process. We found that TGF-b and TNF-a, integral to the initial inflammatory response, inhibit HOXD3 mRNA expression; however, angiogenic cytokines bFGF and IGF-1, more prevalent during the proliferative/granulation phases of wound healing, stimulated HOXD3 mRNA expression. These results indicate that HOXD3 expression is differentially regulated by local cytokine expression.

58B

GENE EXPRESSION PROFILING OF WOUNDED ADULT AND FETAL SKIN USING MICROARRAY ANALYSIS

Presenter: David Chiang
Authors: Chiang D, Colwell AS, Hantash BM, Longaker MT, Lorenz HP

Stanford University School of Medicine

Introduction: We have previously shown that wounded fetal mouse skin heals scarlessly while adult skin scars. The molecular mechanisms underlying this difference remains largely unknown. The objective of this study was to identify the molecular signature of scarless wound healing using microarray analysis.

Methods: Wounds were created on the back of adult, E17, and E19 mice and total RNA was harvested at 1, 12, and 24 hours post-injury. cDNAs were generated using RT-PCR and then hybridized to a gene chip. The data were output into SMD and subjected to regression correlation filtering of ? 0.8, organized by time, age, and wounding status, then analyzed for differential gene expression using SAM.

Results: At 1 hour post-injury of adult mice, we found 54 genes differentially expressed by at least 2-fold compared to unwounded samples. Of these, ubiquitin-activating enzyme E1C demonstrated a 10-fold upregulation and 3 other genes were downregulated more than 5-fold. By 12 hours, 34 genes were differentially expressed by 2-fold, only 3 of which were 5-fold different. At 24 hours, a total of 506 genes were differentially expressed by 2-fold, of which 23 and 3 genes were at least 5- and 10-fold different, respectively. These results as well as similar analyses performed on E17 and E19 skin.

Conclusion: Skin wounding led to dramatic gene expression changes as early as 1 hour post-injury. Fetal skin (E17 and E19) demonstrated a 5- and 10-fold greater number of at least 2-fold differentially expressed genes at 1 hour. We found the gene expression profile exhibited a temporal dependence that was unique to skin age. Although it appears that skin age exerts a significant influence on wound healing response patterns, we discovered an unexpected upregulation of chemokine (C-C motif) ligand 2 in all 3 age groups at various time points studied. Comparisons of response patterns between age groups is ongoing and will allow elucidation of a molecular signature for scarless healing as well as identification of novel gene targets.
THE EVALUATION OF VAC® WOUND FLUID FROM DIABETIC AND NON-DIABETIC WOUNDS: LEVELS OF VEGF, IL-1, EDA-FIBRONECTIN, COLLAGEN, CORRELATION WITH FIBROBLAST MIGRATION

Presenter: Robert Grunfeld, MA
Penn State College of Medicine

Introduction: Vacuum-Assisted Wound Closure, VAC®, is an effective method for expediting the closure of chronic wounds. Diabetic patients with chronic wounds are commonly encountered and are significant cost in the healthcare system. The pathological mechanism underlying impaired diabetic wound healing and the influence of VAC® treatment on diabetic wounds is unclear. In the current study, the effect of diabetic and non-diabetic VAC® wound fluid upon human dermal fibroblast migration is correlated with the synthesis of collagen, fibronectin and cytokines.

Methods: VAC®-treated wound fluid was collected from 24 non-Diabetic (non-DM) (98 collections) and 18 Diabetic wounds (DM) (77 collections). Collections were made over an average period of 7 to 10 days. For the final analyses only patients with chronic lower leg wounds were compared, including 8 non-DM (40 collections) and 10 DM (46 collections). Cell migration studies were executed using human dermal fibroblasts. EDA-Fibronectin and collagen type I levels were determined by Dot-Blot and Western blot analysis. ELISA immunoassay measured IL-1 Beta and Vascular Endothelial Growth Factor (VEGF).

Results: DM wounds exhibited greater levels of IL-1 and VEGF (p<0.05), diminished cell migration, and decreased fibronectin levels as compared to non-DM (p <0.5) wounds. Diabetic wound fluids expressed lower levels of wound protein (fibronectin or collagen), as compared to non-DM wounds over the treatment course.

Conclusions: This study corroborates previous findings regarding higher levels of IL-1 and lower collagen levels in DM wounds. However, higher VEGF levels and diminished cell migration in DM wounds have not been previously reported. Depressed fibroblast function is evidenced by lower cell migration and lower protein synthesis. On the other hand, elevated cytokine levels may reflect an attempt by the wound milieu to promote wound closure. These findings add to the growing body of knowledge in elucidating impaired wound healing in diabetic wounds. Additional research is underway to further study the basic physiology in diabetic wound healing.

CALRETICULIN STIMULATES PROLIFERATION AND MIGRATION OF DIABETIC CELLS TO IMPROVE CUTANEOUS WOUND HEALING

Presenter: Matthew R. Greives, BA
Authors: Greives MR, Cadacio CL, Blechman KM, Rahman M, Levine JP, Gold LI
New York University Medical Center

Introduction: Defective wound healing with consequential morbidities has become an increasingly serious clinical problem in urgent need of novel therapies. Calreticulin, an ER chaperone protein, has shown initial promise as a topical treatment to enhance wound healing.

Methods: We analyzed the effect of CRT in repair of excisional wounds in diabetic mice (lep-/lep-) and compared normal and diabetic wound cells in in-vitro migration and proliferation assays. CRT (50 µg/day for 4 days) was applied to dorsal wounds (6 mm) that were splinted open to prevent contraction, the mice injected with BrDU, and the wounds harvested 3, 7, 10, 14, and 28 days post-wounding.

Results: Time to closure of the diabetic wounds was decreased with CRT (day 17 vs. 21 p<0.05) with a remarkable appearance of dermal appendages at day 28 that were lacking in the untreated controls. Accordingly, epithelial gap was reduced at days 7 and 10 (p=0.05) and granulation tissue was markedly increased at day 7 (p=0.0006). Histologically, the CRT-treated wounds appeared highly cellular with increased BrDU positive proliferating basal keratinocytes and fibroblasts (p=0.05). By picrosirius red staining, increased collagen organization was observed. In-vitro, CRT induced chemotaxis of human fibroblasts, keratinocytes and macrophages with maximal induction at 100ng/ml, 10pg/ml and 1ng/ml, respectively, and greater than positive controls (p<0.05). Importantly, whereas the diabetic cellular counterparts exhibited decreased migration of positive controls, CRT partially restored their migratory capacity. Furthermore, CRT (100 pg/ml) maximally induced proliferation of keratinocytes and fibroblasts by 2.2-fold and 8.3-fold, respectively over the untreated controls.

Conclusion: While the in-vivo effects of CRT show substantial improvement in all aspects of diabetic wound healing, this study elucidated that this process is mediated though increased proliferation and migration of the stromal and epithelial cells. We conclude that CRT has the potential to be a powerful topical therapeutic for the treatment of diabetic wounds through multiple biological effects.
Methods: Human dermal inhibition of myosin IIB prevents collagen matrix contraction. This study was to demonstrate that specific, dose dependent inhibition of myosin IIB prevents collagen matrix contraction.

Introduction: Fibroblasts and myofibroblasts mediate wound contraction and scar contracture by reorganizing and compacting the extracellular matrix. Rho kinase and myosin light chain kinase putatively regulate collagen remodeling. This study used a fixed collagen lattice to reorganize and compact the extracellular matrix. Rho kinase and myosin light chain kinase putatively regulate collagen remodeling by stimulating myosin IIB contractility. The purpose of this study was to demonstrate that specific, dose dependent inhibition of myosin IIB prevents collagen matrix contraction.

Results: Blebbistatin significantly inhibited free-floating and fixed collagen lattice contraction in a dose-dependent manner (p<.05). Removal of blebbistatin restored wild type contraction. Blebbistatin inhibition was conserved despite doubling cell density, collagen concentration, or serum concentration. Cell proliferation was similar in wild type and blebbistatin treated lattices and there was no cell death. Inhibition of myosin IIB reversibly diminished actin stress fiber formation and prevented alpha-smooth muscle actin expression.

Conclusion: The presented results indicate that selective inhibition of myosin IIB prevents fibroblast populated collagen matrix contraction in a dose-dependent, reversible fashion. Myosin IIB is a potent mediator of collagen matrix reorganization irrespective of collagen concentration, serum concentration and cell density. Blockade of myosin IIB activity prevents myofibroblast formation. Myosin IIB may likely be the primary motor protein in fibroblasts that regulates wound contraction and scar contracture.

62B ABSENCE OF HYPERTROPHIC SCAR FORMATION IN IMMUNODEFICIENT MICE

Introduction: We previously demonstrated that application of mechanical strain to a healing wound was sufficient to produce hypertrophic scar (HS) in mice with all the features characteristic of human HS. The exact pathophysiology of HS formation still remains unknown however immunological factors have been suggested as playing a central role in the disruption of the normal processes of wound healing and tissue remodeling. Mammalian fetuses can regenerate and scarlessly heal skin incisions up to the third trimester of gestation, the time when T-lymphocytes appear. It has also been shown that continued antigenic stimulation may lead to excessive fibrosis via activation of T lymphocytes that secret a Fibroblast Activating Factor. In our study we examined the role of T-lymphocytes in the HS formation using congenitally athymic nude mice (impaired T cell system) and specifically identified the cell types and cytokines that are affected by mechanical strain.

Methods: Paired incisions were created on Wild type (WT) and nude mice (n=20) and mechanical strain was selectively applied to achieve tension levels normally experienced by human HS. Scars were harvested after 2-4 weeks and area was determined. Histological examination included H/E, Sirius red, Dapi and markers for inflammatory cells, fibroblasts and vascular cells.

Results: Mechanically strained scars in WT controls at week 2 showed a 10-fold difference in cross sectional area and a 4-fold increase in cellular density between the unstrained and strained scar with all the characteristic features of human HS. Whereas the mechanically strained scars from the nude mice at week 2 appeared almost identical to the unstrained wounds with no quantifiable difference in volume or cellularity. P<0.05

Conclusions: The preliminary results confirm the hypothesis that T-cell deficiency promotes scarless skin repair in mice. Therefore a further understanding of the role of T cells and their derived cytokines on fibroblast function and ultimately the formation of HS would provide a biochemical tool for therapeutic intervention in correcting disorders of scar formation.
ENGINEERED EPIDERMAL GROWTH FACTOR FOR WOUND HEALING APPLICATIONS

Presenter: Daphne P. Ly, MD
Authors: Ly DP, Beck SE, Lee SS, Longaker MT, Cochran JR, Yang GP
Stanford University School of Medicine

Introduction: Epidermal growth factor (EGF) plays a major role in wound healing. Local and sustained presence of EGF has been shown to accelerate wound healing. However, clinical applications for EGF have been limited by its short circulation half-life. Previously, directed evolution was used to engineer EGF mutants (mtEGFs) with 4-30 fold enhanced binding affinity to human EGFR (hEGFR) (JR Cochran, et al., (2006) Protein Eng. Des. Sel. 19, 245-53). We hypothesized that EGFs engineered for enhanced receptor binding affinity would have enhanced biological effectiveness.

Methods: Biologic activity was tested in human and mouse fibroblast lines. Chemotactic migration was measured using a modified Boyden chamber assay in response to serum free medium (SFM), wild-type EGF (wtEGF), and mtEGF after 5 hours. Directional migration was measured using a scratch migration assay and analyzed by inverted phase contrast microscopy. Distance migrated was quantified by percentage of gap closure from that at time zero. All experiments were done in triplicate.

Results: A 2-8 fold increase in chemotactic cell migration was observed in fibroblasts treated with mtEGF compared to those treated with wtEGF. Scratch migration assays showed fibroblasts pulsed with mtEGFs had significantly increased rate of gap closure compared to wtEGF treated cells at 48 hours.

Conclusion: We show that engineered mtEGFs elicit enhanced cell chemotactic and directional migration over wtEGF. These data suggest mtEGFs engineered for increased hEGFR binding affinity have increased functionality compared to wtEGF. This study represents an initial report of growth factors engineered to enhance biological activities for treatment of human disease.

A NOVEL VASCULAR ENDOTHELIAL GROWTH FACTOR ANALOGUE (VEGF-LA) IMPROVES WOUND HEALING IN DIABETIC MICE

Presenter: Sharone M. Jacobs, MD
Authors: Jacobs SM, Trousdale RK, Pollak SV, Julius MA, Simhaee DA, Wu JK, Lustbader JW
Columbia University College of Physicians and Surgeons

Introduction: Delayed wound healing in diabetic patients causes major morbidities, resulting in amputation in approximately 14-24% of chronic diabetic wounds. One cause of delayed healing is impaired angiogenesis. A potent stimulator of angiogenesis is Vascular Endothelial Growth Factor (VEGF). Administration of VEGF has been shown to improve healing; however, its use has been limited by a short half-life. A novel VEGF analogue (VEGF-LA) with a potentially longer half-life and potency was developed in our laboratory using molecular engineering. We hypothesize topical VEGF-LA therapy will improve wound closure rates (WCR) in diabetic healing.

Methods: Full-thickness 2 x 2 cm excisional wounds were created on the dorsa of adult female C57BL/KsJ db/db mice and covered with Tegaderm dressing. The animals were divided into two groups receiving either VEGF-LA (1ug/day; n=10) or vehicle (BSA; n=11) from postoperative days (POD) 1-8. Wound areas were measured on POD 0, 3, 5, 7, 10, 14, and 21 and WCR was calculated. Wound tissues were harvested on POD 21 and stained for Factor VIII and Masson's Trichrome for vessel and collagen content, respectively.

Results: Mice treated with VEGF-LA showed statistically significant improved healing at all time points when compared to BSA up to POD 21 (73.7% vs. 41.7% respectively on POD 21, p=0.0001). VEGF-LA treated wounds had increased granulation tissue formation, collagen deposition, and blood vessel formation (6.2 vs. 2.6 vessels per HPF, p=0.0394) by POD 21 when compared to controls.

Conclusion: Topical application of VEGF-LA significantly accelerates wound healing and increases angiogenesis and granulation tissue formation in diabetic mice compared to controls. Further analysis of harvested wound tissues will elucidate mechanisms by which VEGF contributes to improved healing. Additional studies will be performed to assess the viability and optimal use of hyper-glycosylated proteins for trans-dermal therapy in the clinical setting.
65B
TUMOR-CONDITIONED PLATELET-RICH PLASMA PROMOTES DIABETIC WOUND HEALING: AN UNEXPECTED BENEFIT

Presenter: Giorgio Pietramaggiori, MD
Authors: Pietramaggiori G, Scherer S, Cervi DC, Orgill DP
Brigham and Women’s Hospital, Harvard Medical School

Circulating platelets alter their proteome in the presence of a dormant tumor in vivo. Such changes have been suggested to partake in tumor growth and metastasis. Given the similarities existing between wounds and tumor lesions, and the importance of platelets in wound healing, we hypothesized that tumor-conditioned platelets (TCPs) could effect wound healing.

TCPs were topically applied to full-thickness wounds in diabetic mice bearing two dorsal wounds. Wound closure, contraction, re-epithelialization, vascularization and proliferation were assessed. Comparisons were made between wounds directly treated with TCPs, and untreated control wounds.

A single dose of TCPs accelerated wound closure time (1.7-fold increase), increased granulation tissue formation (8.8-fold increase), vascularity (7.5-fold increase) and cell proliferation (1.7-fold increase) when compared to non-treated wounds of littermate controls on day 10. Mice with a TCPs-treated wound showed intermediate healing responses of their second, non-treated wound, indicative of a host response to donor TCPs in favor of improved healing kinetics.

We present unprecedented findings that TCPs promote wound healing across clinically-relevant parameters. The immanent changes in the platelet proteome of cancer patients supports the notion that genetically engineering platelets or their cellular source, the megakaryocytes, could be a biological tool for the treatment of recalcitrant wound.
Saturday, June 23, 2007

Session 10B
Craniofacial/Bone
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11:00 – 11:50 am

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**66B**

**REGENERATION OF BONE FROM A PERIOSTEUM SEGMENT TRANSPLANTED TO AN ECTOPIC LOCATION**

**Presenter:** Eira S. Roth, BA  
**Authors:** Roth ES, Skoracki RJ, Miller MJ, Rios C, Mathur AB  
**MD Anderson Cancer Center**

**Introduction:** Vascularized autogenous bone grafting is the gold standard technique for the repairation of mandibular bone defects. However, it is an invasive technique that can incur sizable damage to the donor site. Our aim is to show that periosteal tissue can be used as an alternative source of viable osteogenic cells, and that avascular periosteum can be transplanted successfully to an ectopic location where it will synthesize viable bone.

**Methods:** Eight periosteal segments of approximately 3” were removed from the ribs of each sheep and transplanted to the animals’ latissimus dorsi muscle with the cambium layer facing up. Silicone sheeting was secured to the cambium layer to prevent the adhesion of surrounding tissues during wound healing. A sheep was sacrificed at days 2, 4, 6, 21 and 50 following the initial surgery. The lateral thoracic artery was perfused with India ink prior to removal of the tissue samples in order to ascertain the extent of graft revascularization. The samples at all time points were micro-sectioned (4 μm), stained with Hematoxylin and eosin, Movat, and Alcian Blue, and immuno-stained with antibodies for collagen types I, II, III, and Osteocalcin before imaging with light microscopy.

**Results:** Periosteal segments engrafted over the latissimus dorsi muscle showed blood vessel growth across the periosteum-muscle interface by day 4, and full integration at the ectopic site by day 6. While collagen III presented at all time points, collagen II appeared transiently on day 21 indicating the presence of cartilage. Alcian blue staining of glycosaminoglycans surrounding the collagen II region further endorsed the presence of cartilage. Day 21 and 50 stained positive for collagen I and osteocalcin with the presence of mature bone with marrow cavities.

**Conclusion:** Periosteum can be engrafted to an ectopic location successfully. The transferred periosteum segment integrates at the ectopic site and maintains its bone regeneration function for at least 50 days, increasing the bone producing area over time.

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**67B**

**JUGULAR FORAMEN STENOSIS IN CRANIOSYNOSTOTIC RABBITS**

**Presenter:** Mark P. Mooney, PhD  
**Authors:** Mooney MP, Smith DM, Hyre MA, Fellows-Mayle W, Morris JA, Barbano TE, Cooper GM, Vecchione L, Afifi, Fitz CR, Pollack IF, Losee JE  
**University of Pittsburgh**

**Introduction:** Ventriculomegaly, increased ICP, hydrocephalus, and altered venous blood flow have all been reported in humans and rabbits with craniosynostosis. It has been suggested that primary cranial base malformations may stenose jugular foramina, restrict venous outflow, enlarge ventricles, and result in increased ICP and abnormal perfusion patterns. The present study was designed to test this hypothesis by quantifying age-related changes in jugular foramen shape in rabbits synostosis.

**Method:** Data from 124 normal control rabbits (n= 63) and rabbits with familial coronal suture synostosis (n=61) were used in the present study. Rabbits ranged from 10-84 days of age. Younger rabbits were longitudinally CT-scanned in the coronal plane with 1.25 mm cuts and analyzed with Amira. Jugular foramen diameter was taken as the narrowest point on the slice depicting the narrowest complete section of jugular foramen. Skulls of older rabbits were digitally photographed under a Leica stereomicroscope and Northern Eclipse software was used to calculate area, perimeter, diameter, and aspect ratio (shape) of each foramen.

**Results:** On average, the craniosynostotic rabbits had narrower and more asymmetrically shaped jugular foramina than controls at 10, 25, and 42 days of age. In contrast, synostotic rabbits had greater foramen areas and perimeters than controls at 84 days of age. A 2 x 4 (group by age), two-way ANOVA revealed significant group (F=12.9; p<0.001) and age (F=7.2; p<0.01) effects. Craniosynostotic rabbits had significantly (p<0.05) smaller foramen diameters at 25 and 42 days of age. No significant group differences were noted at 10 and 84 days of age.

**Conclusion:** Results showed that jugular foramen diameter in synostotic rabbits was similar in perinates and changed in shape and size in the postnatal period. These findings suggest that jugular foramen stenosis is a compensatory change, secondary to neurocapsular displacement into the posterior cranial fossa. Such stenosis and restricted venous outflow may also be responsible for the altered cerebral flow, ventriculomegaly, and increased ICP noted clinically.
INDIAN HEDGEHOG IS REQUIRED FOR NORMAL DEVELOPMENT OF INTRAMEMBRANOUS CALVARIA

Presenter: Kelly A. Lenton, PhD
Authors: Lenton KA, Manu A, Helms JA, Longaker MT
Stanford University

Introduction: Indian Hedgehog (Ihh) function is essential in endochondral ossification where it coordinates the growth and differentiation of chondrocytes. Intramembranous ossification of the calvarial bones occurs by direct differentiation of mesenchymal cells to osteoblasts without a cartilaginous intermediate. This study provides the first analysis of Ihh expression and function in calvarial bone development.

Methods: Whole skulls were harvested from Ihh-/- mutant mice and wild-type littermates between embryonic day (e)15.5 and postnatal day (p)0. Skulls were used for gross phenotypic analysis, or paraffin embedded and sectioned for histological staining, in situ hybridization and immunohistochemistry. The PtchLacZ mouse provides a reporter for Hedgehog signaling. Ihh-/-;PtchlacZ and Ihh+/+;PtchlacZ skulls were harvested at e15.5 and e16.5, cryopreserved, sectioned and processed for X-gal staining.

Results: LacZ expression indicated significantly reduced Hedgehog signaling in cells lining the intramembranous bones in Ihh-/-;PtchlacZ mice, indicating that Ihh signaling normally occurs in these cells. Comparative analysis of whole skulls and sections of Ihh-/- and wild-type calvaria demonstrated a delay in ossification of both intramembranous and endochondral components of the Ihh-/- skull vault at all stages from e15.5 to p0. The Ihh-/- frontal, parietal, interparietal, and supraoccipital bones were consistently smaller than their wild-type counterparts, and the Ihh-/- interfrontal sagittal and lambda sutures were consistently wider than wild-type sutures. Ihh RNA and protein was localized to proliferating chondrocytes and periosteum associated with the endochondral supraoccipital bone as well as to osteoblasts lining the intramembranous calvarial bones.

Conclusion: This is the first report of Ihh expression and signaling in intramembranous calvarial bones. Together with our phenotypic analysis showing delayed ossification of calvarial bones in Ihh-/- mutant mice, these data support an important role for Ihh in the development of the intramembranous skull vault.

HETEROZYGOTE FGF-R1 GAIN-OF-FUNCTION MUTATION TRANSGENIC MICE HAVE ALTERED CORONAL SUTURE MORPHOLOGY AND ALTERED NOGGIN EXPRESSION

Presenter: Kenton D. Fong, MD
Authors: Fong KD, Lenton KA, Jazayeri L, Warren SM, Longaker MT
Stanford University School of Medicine

Introduction: Syndromal forms of craniosynostosis are associated with FGF-R1 gain-of-function mutations. We have demonstrated that Noggin, a BMP antagonist, is down-regulated by FGF signaling and may play a role in maintaining suture patency. We hypothesize that Noggin expression will be suppressed in FGF-R1 gain-of-function transgenic mice with Pro252Arg mutations (Pfeiffer Syndrome).

Methods: Transgenic Mice expressing LacZ behind the Noggin Promoter were crossbred with Pro252Arg mice to yield offspring with both the Pro252Arg mutation and Noggin/LacZ mutations. The coronal sutures of these animals were compared to control littermates containing only the Noggin/LacZ mutation but both normal FGF-R1 genes. Heterozygotes for the Pro252Arg mutation were used in this study as homozygotes have poorer survival potential. PCR was used to genotype all offspring. In addition, microarray analysis was performed on parietal bone biopsies of Pro252Arg heterozygote animals and their normal littermates to evaluate differences in gene expression.

Results: We found unusual layering of bone in the coronal sutures of our Pro252Arg (+/-)/Noggin/LacZ mice consistent with suture fusion. Although Noggin expression as seen by X-gal staining was not abolished, there was attenuated expression within the suture mesenchyme. Gene expression analysis confirmed down-regulation of noggin in the parietal bones and overall more mature/differentiated expression pattern in the Pro-252Arg (+/-) mice compared to controls.

Conclusion: We successfully evaluated Noggin expression in a transgenic mouse model of Pfeiffer syndrome. This study demonstrates that mice containing FGF-R1 gain-of-function mutations have decreased, but not abolished coronal suture Noggin expression with associated abnormal bone formation.
**70B**

**FGFR2 MUTATIONS IN NEONATAL DURAL CELLS INFLUENCE OSTEODIFFERENTIATION OF MESENCHYMAL STEM CELLS**

**Presenter:** Brian U. Ang, MD  
**Authors:** Ang BU, Spivak RM, Horn RB, Nah HD, Kirschner RE

Children’s Hospital of Philadelphia

**Introduction:** Activating mutations of Fibroblast growth factor receptor (FGFR) 2 (e.g. P253R, C278F) lead to premature cranial suture fusion such as Apert (P253R) and Crouzon (C278F) syndromes. The dura mater plays a critical role in formation and maintenance of cranial sutures; however, its role in syndromic craniosynostosis remains unclear. We previously reported that dura mediates suture patency by secreting soluble factors, inhibiting osteodifferentiation of mesenchymal stem cells (MSCs) in a co-culture system. This study examines the influence of FGFR2 mutations (P253R and C278F) in dural cells on undifferentiated MSCs in co-culture.

**Methods:** Dural cells were harvested from N6 mice and grown to near confluence. Bone marrow-derived MSCs were prepared from 6 week old mouse femurs. Mutant (P253R and C278F) and wild-type FGFR2 constructs were subcloned into adenoviral vectors. Dural cells were infected with adenovirus and protein expression was confirmed by immunostaining. Infected dural cells were co-cultured with MSCs using a transwell system. MSCs co-cultured with empty transwell inserts served as a control. MSC RNA was extracted on days 7 and 14, and RNA expression of Runx2 (early marker of osteodifferentiation), osteopontin (OP), alkaline phosphatase (AP), and bone sialoprotein (BSP, a late marker) was determined by Northern blotting.

**Results:** MSCs co-cultured with P253R- and C278F-FGFR2 expressing dural cells showed an increase in Runx2, AP, and OP expression by day 7, versus controls. By day 14, there was a further increase in AP, while Runx2 and OP levels decreased. BSP expression did not increase for all groups. Higher AP and Runx2 expression in MSCs co-cultured with P253R-FGFR2 dural cells than MSCs co-cultured with C278F-FGFR2 dural cells indicated increased osteodifferentiation.

**Conclusion:** P253R- and C278F-FGFR2 mutations in neonatal dural cells promote osteodifferentiation of MSCs in co-culture. P253R-FGFR2 expressing dural cells were more effective in stimulating osteodifferentiation, suggesting an increased maturation rate of preosteoblastic cells in Apert syndromic craniosynostosis.

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**71B**

**NUCLEAR TRANSLOCATION OF STAT1 INDUCES UPRREGULATION OF SMAD7 IN FIBROBLASTS ISOLATED FROM DUPUYTREN'S DISEASE – WHAT IS THE RELEVANCE OF THE IFNGAMMA-TGF-BETA1-CROSSTALK DURING FIBROGENESIS OF THE HAND**

**Presenter:** Juergen Kopp, MD, PhD  
**Authors:** Kopp J, Seyhan H, Akkermann O, Simon H, Horch RE

Chirurgische Universitaetsklinik

**Introduction:** In Dupuytren’s disease Smad mediated TGF-beta1 specific signaltransduction plays a key role during induction and regulation of this fibroproliferative disorder. Dysregulation of this autoregulative system results in overexpression of endogenously expressed TGF-beta1. Subsequently, an autoinductive loop with consecutive overexpression of profibrotic proteins is established. The aim of our experiments was the indirect overexpression of inhibitory acting Smad7 via manipulation of the IFNgamma-TGF-beta1-crosstalk on the cellular and nuclear level to reduce endogenous TGF-beta1 expression levels.

**Methods:** Fibroblasts were isolated and amplified from tissues obtained either by partial or total aponeurectomies of affected palm fascias by explant culture. Cells were incubated on chamberslides. After 24 h one part of the cells was stimulated by application of recombinant TGF-beta1 or IFNgamma, other cells were infected with adenoviral Smad7. Starved fibroblasts, standard cultivated cells and fibroblasts transfected with STAT1-siRNA were used as controls. For morphologic investigation of the IFNgamma-TGF-beta1-crosstalk results in a suppression of profibrotic effects of TGF-beta1. Thereby a new molecular basis for future strategies in the treatment of surgically relevant fibroproliferative disorders is provided.
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