1
A Cost-Utility Analysis Comparing Oncoplastic Breast Surgery with Standard Lumpectomy in Large Breasted Women

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PURPOSE: Scarce cost analysis exists comparing oncoplastic breast surgery (OBS) with standard lumpectomy (SL). Our goal was to perform a cost-utility analysis comparing OBS with SL for breast cancer in the large breasted patient.

METHODS: Cost-utility methodology involved a systematic literature review compiling outcomes and their probabilities for the treatment of unilateral breast cancer using either oncoplastic resection/reconstruction with contralateral symmetry operations or unilateral lumpectomy operations. Utility score surveys were used for each outcome to estimate quality-adjusted life years. Medicare payment data represented costs. A decision analysis tree and incremental cost-utility ratio analysis portrayed the more cost-effective strategy. Sensitivity analyses were performed.

RESULTS: The literature review noted that OBS led to fewer positive margins compared with the SL (10% vs 18%). Utility scores for a successful operation favored the OBS patients (92.6 vs 86.55) but for positive margins favored SL patients (74.2 vs 70.2). OBS costs more than SL ($6782.36 vs $2399.99). Decision tree analysis revealed that OBS was more cost-effective with an incremental cost-utility ratio of $2473.54/quality-adjusted life years. Sensitivity analysis noted that SL became cost-effective when obtaining a utility score for successful surgery of greater than 92.33 (vs its surveyed value of 86.55).

CONCLUSIONS: OBS in the large breasted patient provides a cost-effective treatment option when compared with SL and should be considered as a primary treatment option.

2
T Cells Are the Primary Source of TGF-β1 in Lymphedema

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PURPOSE: We have shown previously that transforming growth factor (TGF)-β1 plays a critical fibrogenic role in lymphedema. The purpose of this study was to determine the cellular source(s) of TGF-β1, understand the effects of TGF-β1 on lymphatic endothelial cells (LECs), and investigate the efficacy of a novel anti-TGF-β1 compound in preclinical mouse models of lymphedema.

METHODS: We developed 3 distinct transgenic mice with selective knockout of TGF-β1 from T or myeloid cells and a non-functional TGF-β receptor on LECs. These mice underwent microsurgical lymphatic ligation in the tail or popliteal lymphadenectomy. To study the efficacy of our novel anti-TGF-β1 compound, we compared this treatment with control mice after lymphatic injury.

RESULTS: TGF-β1 production was significantly decreased in T cell but not myeloid TGF-β1 knockout transgenic mice (50% reduction; $P < 0.01$). Furthermore, TGF-β1 knockout T-cell transgenic mice displayed decreased fibrosis, inflammation, and adipose deposition compared with controls or TGF-β1 knockout myeloid mice. Blockade of TGF-β1 activity in LECs had no effect on the pathological changes of lymphedema but did increase lymphangiogenesis. Systemic or topical application of a novel anti-TGF-β1 compound decreased fibrosis, improved lymphatic function, and reversed the established swelling in the tail model.

CONCLUSIONS: We have shown for the first time that T cells are the primary source of TGF-β1 in lymphedema and that inhibition with a novel anti-TGF-β1 compound potently decreases tissue fibrosis after lymphatic injury. Furthermore, we have shown that the primary mechanism in which TGF-β1 causes lymphatic dysfunction is because of tissue fibrosis rather than the direct effects on LECs.

3
Mechanisms of Obesity-Related Lymphatic Dysfunction

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PURPOSE: Lymphatic endothelial cell (LEC) function is regulated by the activation of VEGFR-3 by VEGF-C, and recent studies suggest that this signaling pathway is disturbed in obesity. Therefore, the purpose of this study was to develop a transgenic mouse model to selectively examine the effects of VEGFR-3 signaling in the lymphatic system and to use this model to understand how lymphatic defects modulate metabolic syndrome in obesity.

METHODS: To activate VEGFR-3 signaling in LECs independent of VEGF-C, we mated FLT4-Cre mice with PTEN-LoxP mice. Because FLT4 is an LEC-specific promoter, activation of Cre with tamoxifen results in knockout of PTEN, an intracellular inhibitor of VEGFR3, only in LECs thereby increasing the activation of VEGFR-3 downstream pathways. We used this model to analyze lymphatic function and metabolic parameters in a high-fat diet model of obesity.

RESULTS: PTEN knockout in LECs markedly increased lymphatic function in response to inflammation. More importantly, the loss of PTEN in obese mice significantly decreased the deleterious effects of obesity on the lymphatic system compared with controls. Moreover, even though PTEN knockout mice became obese when fed a high-fat diet, these mice were protected from metabolic syndrome and hepatic steatosis suggesting that lymphatic function is a significant regulator of these outcomes after weight gain.

CONCLUSIONS: We have shown that impaired VEGF-C/VEGFR-3 signaling in LECs is a major regulator of obesity-induced lymphatic dysfunction. More importantly, we have shown that increasing downstream signaling pathways of VEGFR-3 only in LECs significantly decreases metabolic syndrome and hepatic steatosis.

4
Lymph Node Transfer Decreases Swelling and Restores Immune Function in a Transgenic Model of Lymphedema

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PURPOSE: Lymphedema is a morbid disease, and lymph node transfer (LNT) is considered the most promising treatment. The mechanism of LNT remains unclear, and its effectiveness in restoring immune function is not well explored. Therefore, the purpose of this study was to analyze lymphatic and immune function after LNT.

METHODS: Hindlimb lymphedema was induced in a transgenic mouse model by ablating the lymphatic system using diphtheria toxin. Two weeks later, experimental animals underwent popliteal LNT, whereas controls were treated with popliteal incision alone. Limb swelling, immune cell trafficking, and immune responses were then analyzed 8 weeks later.

RESULTS: LNT animals had a marked (85%) reduction in foot swelling from baseline (ie, 2 weeks after lymphatic ablation); in contrast, control animals had no improvement in swelling during this period of time. These measurements were confirmed with histological studies demonstrating a more than 90% increase in adipose deposition in the control animals compared with LNT. In addition, animals treated with LNT had decreased infiltration of T cells and marked increase in lymphangiogenesis and lymphatic regeneration. Moreover, animals treated with LNT were able to traffic dendritic cells to their lymph nodes (426% increase) and had significant increases in antibody responses compared with controls. In response to DNFB, systemic immune responses were restored in LNT group.

CONCLUSIONS: LNT markedly induces lymphangiogenesis and improves lymphatic function in a mouse model of lymphedema. Transplanted lymph nodes maintain immunologic function and can support migration of antigen-presenting cells.

5
A Novel Implantable Angiogenic Nanotechnology Improves Bone Mineralization, Biomechanical Strength, and Union Rates in Irradiated Fractures

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PURPOSE: Pathologic fractures and associated nonunions arising in irradiated bone are complex management dilemmas for reconstructive surgeons. We developed an implantable, sustained-release nanoparticle formulation of a known angiogenic small molecule, deferoxamine (DFO) that obviates the need for serial injections of standard DFO. Here, we investigate the efficacy of nano-DFO compared with standard DFO in its ability to improve metrics of mineralization, mechanical strength, and bony union.

METHODS: Rats (n = 44) were divided into 4 groups: fracture, radiated fracture, radiated fracture with standard DFO, and radiated fracture with nano-DFO. Radiated groups received radiotherapy 2 weeks before mandibular osteotomy. The nano-DFO group received implantation of the drug at the time of surgery. After a 40-day healing period, mandibles were assessed for bony union, imaged with micro-computed tomography, and mechanically tested to failure. Analysis of variance was used for comparison ($P < 0.05$).

RESULTS: We observed decreases in all metrics for the radiation group that were remediated with the addition of both DFO and nano-DFO therapies. For metrics of bone mineral density, total mineral density, bone volume fraction, stiffness, and failure load, there was no difference between the 2 treatments. However, there was a clinically relevant increase in bony unions with nano-DFO therapy that was 24% higher than standard DFO (67% vs 91%).

CONCLUSIONS: Our data demonstrate in vivo efficacy for the mineralization and biomechanical properties of implanted nano-DFO when compared with normal DFO. We support the continued investigation of this promising treatment in its translation for the management of pathologic fractures and associated nonunions after radiotherapy.

6
Targeting the Signaling Pathway of a Multipotent Connective Tissue Stem Cell to Treat Heterotopic Ossification and Muscle Fibrosis

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PURPOSE: Heterotopic ossification (HO) occurs with severe trauma causing significant functional limitations. Identification of progenitor cells and signaling mechanisms that cause these processes is critical for identifying therapeutic options.

METHODS: C57Bl/6 mice underwent Achilles' tenotomy and 30% TBSA burn to induce HO. Immunostaining and mRNA quantification were performed for the tendon lineage marker, *Scleraxis*. Separately, mice expressing hyperactive bone morphogenetic protein receptor (*Scx-creERT/caACVR1$^{fl/fl}$*) were generated and they received cardiotoxin to induce intramuscular HO followed by histologic analysis after 3 weeks. Given its known effects on tendon, ciprofloxacin was used to treat mesenchymal cells in vitro followed by mRNA quantification of *Scx* and osteogenic differentiation assays. Finally, mice treated with control or ciprofloxacin (10 mg/kg) received burn/tenotomy followed by micro-computed tomography after 9 weeks (n = 4/group).

RESULTS: Burn/tenotomy increased Scleraxis expression at the tenotomy site (A). *Scleraxis* lineage cells with hyperactive bone morphogenetic protein receptor activity were sufficient to form intramuscular HO after trauma (B). Ciprofloxacin significantly reduced *Scleraxis* gene expression in vitro, with a corresponding decrease in osteogenic differentiation (C). Treatment of burn/tenotomy mice with ciprofloxacin significantly reduced HO after 9 weeks (8.50 v 3.67 mm^3, $P < 0.05$; D).

CONCLUSIONS: Muscle- and tendon-resident progenitor cells marked by expression of *Scleraxis*, a tendon lineage marker, are capable of forming cartilage and osseous lesions. Targeted inhibition of *Scleraxis* gene expression using ciprofloxacin, a readily available therapeutic is a powerful treatment for patients at risk for muscle fibrosis and HO.

7
Does Antimicrobial Irrigation of Breast Implant Pockets Reduce Capsular Contracture? A Systematic Review and Meta-Analysis

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PURPOSE: The purpose of this study was to conduct a meta-analysis to determine whether irrigation of breast implant pockets with antimicrobial agents reduces the rate of capsular contracture. Capsular contracture is the most common complication after primary augmentation mammoplasty, yet its etiology remains cryptogenic.

METHODS: PubMed was searched for publications from January 1, 2000, through October 2015. Studies with the following criteria were included: primary breast augmentation with implants, use of antimicrobial irrigation, and documentation of capsular contracture. Our primary outcome was incidence of capsular contracture. The quality of included studies was assessed independently. Studies were meta-analyzed to obtain a pooled odds ratio (OR) describing the effect of antimicrobial irrigation on capsular contracture.

RESULTS: The meta-analysis included 8 studies and totaled 8538 patients. Five thousand two hundred fifty-five patients received antimicrobial irrigation, and 3544 patients did not. Analysis revealed that combined antimicrobial irrigation, the antibiotic irrigation subgroup, and iodine subgroup were associated with an increased incidence of capsular contracture [OR, 2.60; 95% (confidence interval) CI, 2.3–2.94; OR, 1.41; 95% CI, 1.17–1.70; OR, 3.19; 95% CI, 2.23–4.56; $P < 0.00001$; $I^2 = 99.9$], respectively.

CONCLUSIONS: Antimicrobial irrigation of implant pockets does not reduce the incidence of capsular contracture. The authors recommend that further prospective multicenter trials be conducted to further elucidate the role of antibiotic irrigation in capsular contracture.

8
Activation of HIF by Small-Molecule Inhibitors of PHD2 Improves Healing of Cutaneous Wounds and Calvarial Defects

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PURPOSE: Impaired healing of wounds and cranial defects represents a significant clinical challenge. Hypoxia-inducible factor (HIF), master regulator of cellular response to hypoxia, is critical for enhancing the appropriate inflammatory and angiogenic responses that promote the healing of both wounds and skeletal defects. Herein, we examine the effect of small molecule activators of the HIF pathway on both of these processes in vivo.

METHODS: We generated 25 small-molecule analogue inhibitors of PHD2. A high-throughput HRE-luciferase assay was performed on NIH 3T3 fibroblasts to identify compounds that would achieve the greatest increase in the HIF pathway. The best 3 compounds were tested in vivo using a murine model of cutaneous wound and cranial defect healing with splinted 6-mm full-thickness excisional wounds and 2-mm frontal and parietal calvarial defects, respectively.

RESULTS: By using the HRE-luciferase assay, we identified compounds GPHD-11, GPHD-14, and GPHD-15 for upregulating HIF activity 4.01-, 4.38-, and 4.08-fold, respectively ($^*P < 0.05$). Full-thickness excisional wounds treated with GPHD-11, GPHD-14, and GPHD-15 completely healed on days 11.5, 11.4, and 11.8, respectively, versus day 13.4 for controls ($^*P < 0.05$). Cranial defects in both frontal and parietal regions all healed significantly more with small molecule treatment ($^*P < 0.05$).

CONCLUSIONS: Our results validate the ability of small-molecule analog inhibitors of PHD2 to activate the HIF pathway in vitro. In vivo, we demonstrate accelerated healing or cutaneous wounds and cranial defects with application of our compounds. With further studies, the small-molecule activators of HIF may prove to be a novel therapeutic.

9
Solving the Challenge of Cell Sourcing for the Translation of Patient-Specific Tissue-Engineered Ears

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PURPOSE: Previously, we fabricated patient-specific auricles using bovine auricular chondrocytes, which displayed effective permanence with structural, biochemical, and mechanical properties similar to native auricular cartilage after 6 months in vivo. To achieve clinical translation, we must surmount the large 250 million human auricular chondrocytes (hAuCs) requirement. Thus, we sought to generate human auricular cartilage through the combined implantation of hAuCs with human mesenchymal stem cells (hMSCs) as a novel cell sourcing strategy.

METHODS: hAuCs from discarded otoplasty specimens and bone marrow–derived hMSCs were encapsulated into type I collagen hydrogels in ratios of 100:0, 50:50, and 0:100 hAuCs:hMSCs with a cell density of 25 million cells/mL hydrogel. The 8-mm diameter constructs were implanted subcutaneously in the dorsa of nude mice for 1 and 3 months.

RESULTS: Constructs containing 100% hAuCs or 50:50 hAuCs:hMSCs maintained cylindrical geometry and white cartilage-like appearance, whereas hMSCs contracted. hAuCs:hMSCs scaffolds developed an auricular cartilage microstructure, including organized perichondrium composed of collagen, rich proteoglycan, cellular lacunae, and a dense elastin fibers network. Biochemical analysis confirmed that mixed cell constructs featured significantly more proteoglycan content than the 100% hMSCs group; proteoglycan content increased significantly between 1 and 3 months.

CONCLUSIONS: Coimplantation of hAuCs with hMSCs in a 50:50 ration produces human auricular cartilage that is indistinguishable from native auricular cartilage. This approach reduces the autologous auricular chondrocyte requirement to clinically obtainable numbers (~1 mg of donor ear cartilage) making this a viable strategy for the generation of patient-specific, high fidelity tissue-engineered ears.

10
Platelet Function Testing Shows Promise for Predicting Thrombosis and Therapeutic Monitoring in Microsurgery: Results of a Prospective Trial

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PURPOSE: Although thrombotic complications are devastating, reconstructive microsurgeons lack a useful means to quantify the thrombotic potential in hemostatic pathways. Impedance aggregrometry (IA) is a novel means to rapidly measure platelet function, with previously demonstrated utility in interventional cardiology. We investigated its microsurgical application as an early predictor of thrombotic flap complications and its utility in monitoring antiplatelet therapy.

METHODS: After obtaining institutional review board approval, consecutive patients undergoing microsurgical flap reconstruction were enrolled in our prospective observational study. Serial phlebotomy was performed preoperatively and postoperatively after standardized administration of aspirin. We performed Multiplate IA (Roche/Genentech, South San Francisco, Calif.) utilizing various platelet agonists as well as complete blood count and conventional coagulation (PT/PTT) testing on all samples. Outcomes included thrombosis, reoperation, and significant bleeding postoperatively. Analyses were performed with Stata version 14 with Student's *t* and Fisher's exact tests.

RESULTS: Interim analyses were performed after enrollment of 20 subjects. Six subjects experienced intraoperative or postoperative anastomotic thromboses not attributable to surgical technique. These patients were younger (40 vs 56.6 years, $P = 0.021$) but were otherwise well matched. Platelet aggregation to the arachidonic acid agonist demonstrated decreased response (mean change, −13.8 vs −33.1 AU, $P = 0.031$) and greater residual platelet activity (mean, 46.1 vs 20.7 AU, $P = 0.032$) in the thrombosis group despite standard aspirin treatment.

CONCLUSIONS: IA identifies a thrombotic flap phenotype, characterized by high-residual postoperative platelet activity and low response to antiplatelet therapy. Platelet function monitoring has significant potential to predict thrombotic risk and drive individualized anticoagulation to prevent flap loss.

11
Noncanonical BMP Signaling Regulates Endochondral Bone Development and Trauma-Induced Heterotopic Ossification

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PURPOSE: Canonical bone morphogenetic protein signaling plays a central role in endochondral bone development and trauma-induced heterotopic ossification (HO). However, the role of noncanonical bone morphogenetic protein signaling through the transforming growth factor-activated kinase (TAK1) pathway has not been evaluated in HO. We hypothesize that the TAK1 pathway is crucial for endochondral bone development and HO.

METHODS: Cre-conditional TAK1 knockout mice (*Prx1-Cre;Tak1$^{fl/fl}$) growth plates were evaluated during early postnatal development (P3-P8).* Tamoxifen inducible TAK1 knockout (*Ubi.CreERT*;*Tak1$^{fl/fl}$*) and *Prx1-Cre;Tak1$^{fl/fl}$* mice and *littermate controls* underwent Achilles' tenotomy with 30% TBSA. Micro-computed tomography imaging, histological analysis, osteogenic differentiation and RNA/protein analysis were performed to assess the TAK1 pathway in the cartilaginous HO.

RESULTS: *Ubi.CreERT*;*Tak1$^{fl/fl}$* mice formed 30% less ectopic bone compared with control 9 weeks after burn/tenotomy ($P < 0.05$) (A). *Prx1-Cre;Tak1$^{fl/fl}$* mice exhibit smaller tibia bone size, more immature proliferating chondrocytes, and disorganization of mature bone. Mesenchymal cells isolated from tamoxifen-inducible Tak1 knockout mice showed decreased osteogenic and chondrogenic potential compared with cells from littermates (B and C). In vitro inhibition with TAK1 inhibitor NG-25 similarly significantly reduced chondrogenic and osteogenic differentiation ($P < 0.05$) (D).

CONCLUSIONS: TAK1 plays a prominent role in chondrogenesis during limb development and ectopic bone formation, confirmed by abnormal bone growth and diminished HO in knockout mice. NG-25 appears to be a candidate drug for TAK1 inhibition, which we will evaluate for the treatment of HO using our burn/tenotomy model.

12
Reconstructing Craniofacial Trauma with Fat Grafting: Predictors of Successful Outcomes

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PURPOSE: Craniofacial disfigurement creates psychological distress and functional impairment. Fat grafting improves contour; however, unpredictability of volume retention is a significant limitation.

METHODS: This institutional review board-approved prospective cohort study was funded by the US Department of Defense. Twenty patients with craniofacial deformities underwent fat grafting. Volume retention was evaluated using computed tomography scans. A portion of fat was evaluated for stromal vascular fraction cell type populations by flow cytometry. Quality of life measures were recorded. Five patients underwent a second fat grafting procedure after completing the 9-month follow-up period.

RESULTS: Volume retention stabilized at 3 months and averaged 63% (±16%) at 9 months. The retention at 3 months was significantly predictive of 9 month volume ($P = 0.006$). Higher stromal vascular fraction cell viability was correlated with improved volume retention ($P = 0.008$). Volume retention in the first procedure was predictive of the second operation ($P = 0.05$). Satisfaction with physical appearance ($P = 0.001$) and social functioning quality of life ($P < 0.04$) improved from baseline to 9 months. There were no serious adverse events.

CONCLUSIONS: Fat grafting craniofacial defects is effective in improving volume deficits, with 40% volume loss anticipated. Viability of fat harvested impacts overall retention. The volume retention in subsequent procedures in the same patient had similar volume retention, suggesting a role for innate biologic characteristics of fat tissue in fat graft healing.

13
Creation of a Bioengineered, Porcine, Full-Thickness Skin Graft with a Perfusable Vascular Pedicle

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PURPOSE: Current treatments for skin loss result in contracture, scar, and poor function; therefore, the creation of an autologous full-thickness skin analog remains of paramount importance. The purpose of this project was to create a full-thickness skin analog by perfusion decellularization of a porcine fasciocutaneous flap.

METHODS: Fasciocutaneous porcine flaps were harvested, and perfusion decellularization was performed via vascular pedicle. The resulting matrix was characterized; in vitro biocompatibility and mechanical testing were performed; and regenerative potential was evaluated. Immunological response, biocompatibility, and regenerative potential were assessed using in vivo models.

RESULTS: Perfusion decellularization removed all cellular components with preservation of ECM proteins in a similar composition to native skin. Biaxial testing revealed preserved elastic properties. Immunologic response and biocompatibility assessed via implantation and compared with native xenogenic skin and commercially available dermal substitutes revealed that neovascularization and tissue integration were most optimal for our flap. Composition of infiltrating immune cells was similar to sham and resembled inflammatory phase of healing. Implantation into full-thickness skin defects demonstrated optimal tissue integration and skin regeneration without cicatrization.

CONCLUSIONS: We have developed a protocol for the generation of a full-thickness skin matrix of clinically relevant size, containing a vascular pedicle that can be utilized for perfusion decellularization and, ultimately, anastomosis to recipient vascular system precellularization. We demonstrate formidable regenerative potential and favorable immunological response resulting in optimal tissue integration.

14
Fabrication of a Tissue-Engineered Prevascularized Perfusable Skin Flap

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PURPOSE: Fabrication of tissues with an inherent hierarchical vascular network remains the holy grail of tissue engineering. Herein, we fabricate a prevascularized full-thickness cellularized skin equivalent containing a 3-dimensional vascularized network of interconnected macrochemicals and microchannels lined with vascular cells, within a collagen neodermis containing encapsulated fibroblasts and an epidermis composed of human keratinocytes.

METHODS: Pluronic F127 was used for network preparation: 1.5 mm diameter "U"-shaped macrofibers and 100- to 500-μm interwoven microfibers were heat extruded and then embedded within type I collagen into which CFP-tagged human placental pericytes and human foreskin fibroblasts at a density of 1×10^6 cells/mL, respectively, had been encapsulated. After pluronic sacrifice, channels were intraluminally seeded with 5×10^6 cells/mL RFP-tagged human aortic smooth muscle cells, 5×10^6 cells/mL GFP-tagged human umbilical vein endothelial cells (HUVEC-GFP), and the construct was then topically seeded with 1×10^6 cells/mL human epidermal keratinocytes. Multiphoton microscopy and histology were conducted after 7 and 14 days of culture.

RESULTS: Multiphoton microscopic imaging demonstrates a hierarchical vascular network containing macrovessels and microvessels lined by endothelial and smooth muscle cells and supported by perivascular pericytes, all in appropriate microanatomic arrangement. Neodermal human foreskin fibroblasts proliferated throughout the observation period, and the human epidermal keratinocyte neoepidermis remained stable along the superficial aspect of the construct.

CONCLUSIONS: We have successfully fabricated a tissue-engineered prevascularized full-thickness skin flap construct with stable and anatomically appropriate vascularity. Our platform provides tremendous promise in furthering the development of tissue-engineered skin and other types of prevascularized flaps.

15
Macaques Implanted with Regenerative Peripheral Nerve Interfaces Perform Dexterous Prosthesis Finger Movements

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PURPOSE: Regenerative peripheral nerve interfaces (RPNIs) are promising for interfacing human intentions to myoelectric prostheses. Rat studies led to proof of RPNI long-term function and high signal-to-noise ratio with no adverse biological effects. However, true voluntary fine control of fingers and hand prostheses would be more convincing with macaque implanted RPNIs.

METHODS: Two macaques had RPNIs implanted (n = 3/macaque) in the forearm. The RPNI consists of a free muscle graft implanted on the end of a transected nerve fascicle. Intramuscular electromyogram (EMG) electrodes were implanted in each RPNI. Macaques were trained to perform index finger movements to acquire virtual targets on a computer screen. Finger position was recorded via a flex sensor on the index finger.

RESULTS: At harvest, RPNIs were well vascularized but smaller in size than when implanted. For the continuous EMG decode using 10-fold cross-validation, the resulting predicted finger position had a correlation coefficient $\rho = 0.82$ between predicted and true finger positions. The EMG decode correctly classified 97.7% of movements (of 261 total movements). RPNI muscle fibers were continuing to regenerate after implantation for 1 year.

CONCLUSIONS: Macaques voluntarily controlled virtual finger movements with signals transferred through implanted RPNIs.

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A Preclinical Swine Model for Whole Eyeball Transplantation: Planning and Procedural Aspects

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PURPOSE: Whole eyeball transplantation (WET) is the holy grail of vision restoration and is conceptually the most challenging of vascularized composite allografts. The swine eye is analogous to the human eye and is the ideal model for human WET. Our goal was to develop the protocols (surgical planning/procedures/post operative imaging /evaluations) as a foundation for a robust, large animal, preclinical WET model.

METHODS: WET techniques were optimized in 17 fresh tissue swine dissections. An eyeball-periorbital vascularized composite allograft subunit with extraocular muscles, and optic nerve (ON) was raised superolaterally and anastomosed to the recipient external ophthalmic artery after exenteration. Methylene blue perfusion and microfil vascular mapping of central retinal artery/vortex veins/ciliary plexus was done. Orbital contents and ON were imaged with DCE-DTI-magnetic resonance imaging (T1/T2 MRI at 3T/7T/9.4 Tesla). Advanced protocols for histopathology, immunohistochemistry, epoxy embedding, corrosion casting, and optical coherence tomography/tonometry/fundoscopy/ERG were optimized, and surgical techniques for ON crush, cut, and coaptation were established.

RESULTS: Like humans, the swine retina is holangiotic, and the ON has a lamina cribrosa. However, the central retinal artery is absent with a large external ophthalmic artery. Optical coherence tomography/MRI allowed real-time, high definition, noninvasive, in situ, micron-scale, cross-sectional visualization of structure/topography of ocular structures.

CONCLUSIONS: Our study is the critical first step toward a swine WET model optimized for viability, retinal survival, ON regeneration, and reintegration while documenting key immune responses and enabling key neuroimmunotherapeutic interventions.

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Engineering a "Hybrid Thymus" to Promote Transplant Tolerance

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PURPOSE: Targeting the process of central (thymic) selection of developing T lymphocytes is the key tolerogenic mechanism of bone marrow transplantation-based protocols of transplant tolerance induction. However, they are not amenable to most transplant recipients. Thymic epithelial cells (TECs), a population of stromal cells residing in the thymus, exerts a major contribution to central selection. However, donor TEC do not develop following bone marrow transplantation protocols. Therefore, we propose a new immunomodulatory strategy based on generating a donor-recipient "hybrid thymus," through donor TEC engraftment, to reengineer the thymic microenvironment and achieve dominant central tolerance.

METHODS: We developed our own protocol for isolating TEC via a combination of negative and positive selection. Purified BALB/c TEC was injected intrathymically into C57BL/6 with or without co-stimulation blockade [cytotoxic T lymphocyte-associated antigen-4 immunoglobulin (CTLA4-Ig)]. Fourteen and 28 days postinjection donor-TEC survival was assessed, and peripheral T cells analyzed for changes in TCR-β Vβ11 expression—a marker of negative selection.

RESULTS: Our optimized purification protocol yields an average 70% TEC purity. As expected, unmanipulated animals promptly rejected TEC. However, CTLA4-Ig co-administration exerted a significant protection. In this latter group, the percentage of Vβ11+ T cells on d24 was significantly lower indicating functional activity of the engrafted donor TEC.

CONCLUSIONS: Our preliminary data show that the thymic engraftment, survival, and function of allogenic TEC can be promoted by CTLA4-Ig. These exciting results indicate that engineering a donor-recipient hybrid thymus is feasible and has the potential to promote a dominant regulation of alloreactivity that could be conducive to transplant tolerance induction.

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Predictors of Surgical Success and Failure in Migraine Surgery

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PURPOSE: Migraine surgery improves symptoms in 68% to 95% of patients. However, predictors of surgical success and failure remain unknown.

METHODS: Forty subjects were prospectively enrolled and completed questionnaires on migraine history, migraine headache index (MHI) [migraine headache (MHA) frequency × duration × pain severity], migraine disability, headache impact test (HIT6), and pain self-efficacy questionnaire. After completing a 12-month follow- up, the "best" outcome patients [MHI 0 (no migraines), n = 11] and "worst" outcome patients (MHI > 100, n = 4) were grouped and analyzed.

RESULTS: Age of MHA onset was significantly higher in patients who failed surgery (37 vs 18.8 years); age at surgery was not significant. MHA duration was higher in non-responders (50 vs 17.8 hours), whereas pain severity and frequency were not. Factors signifying MHA severity (MHI total score, migraine disability, HIT6, and pain self-efficacy questionnaire scores) were not predictors of failure/success.

CONCLUSIONS: When comparing patients with the best and worst outcomes after migraine surgery, increasing age at MHA onset and longer MHA duration are negative predictors. These factors should be considered when screening patients for surgery.

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Identification of Bone-Chondro-Stromal Progenitor Cell Populations within Trauma-Induced Heterotopic Ossification

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PURPOSE: Recently, Longaker et al have identified a bone-chondro-stromal progenitor (BCSP) cell in developing bone and fracture sites, which contribute to normal bone formation. Here, we investigate whether pathologic extraskeletal bone is composed of the cellular components of normal bone with attention to BCSPs.

METHODS: C57BL/6 mice underwent 30% TBSA partial-thickness burn with Achilles' tenotomy. Mice were euthanized 3 weeks postinjury, and tenotomy site was harvested and digested for FACS to quantify BCSPs (AlphaV+/CD105+/Tie2-/CD45-/Thy1-/6C3-). Immunostaining was performed to localize BCSPs in trauma sites. Subsequently, BCSPs were isolated from neonatal mice with ubiquitous tdTomato expression using FACS and transplanted into burn/tenotomy recipients at the tenotomy site to identify the phases of HO to which these cells contribute.

RESULTS: BCSPs were noted on flow cytometry in sites of developing HO (A and B). No enrichment of BCSPs was noted in the uninjured hindlimb after injury. Immunostaining confirmed the presence of BCSPs within developing HO at the tenotomy site (C). When transplanted into the burn/tenotomy site, these cells integrate into the cartilage and osseous phases of HO (D).

CONCLUSIONS: Our study confirms that a previously identified population of BCSPs contribute to pathologic, ectopic bone. These findings suggest that HO exhibits qualities similar to normal, developing or healing bone. Studies are underway to determine whether the presence of these cells can serve as an early marker for HO formation.

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Does Intraoperative Use of Vasopressors Increase the Risk of Flap Thrombosis?

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PURPOSE: Most microsurgeons anecdotally avoid the use of vasopressors during free flap surgery. The purpose of this study was to examine their effects on free flap outcomes.

METHODS: All free flap reconstructions from 2004 to 2014 were reviewed. Vasopressors were given intraoperatively when blood pressure dropped more than 20% from the preoperative baseline. The timing of intraoperative vasopressor administration was divided into 3 phases: from anesthesia induction to 30 minutes before the start of flap ischemia (P1), end of P1 to 30 minutes after the end of ischemia (P2), and end of P2 to end of surgery (P3). Three types of vasopressors were used: phenylephrine, ephedrine, and calcium chloride.

RESULTS: A total of 5129 free flap cases for head and neck, breast, trunk, and extremity reconstructions were identified. The incidences of intraoperative and postoperative pedicle thrombosis (including both arterial and venous) were 0.4% and 3.4%, respectively. Total flap loss was 1.8%. Vasopressor use during P1, P2, and P3 were 72%, 37%, and 28%, respectively. Use of any vasopressors during P1, P2, and P3 had no effect on intraoperative or postoperative pedicle thrombosis or flap loss (odds ratio = 0.96, $P = 0.79$). Further analysis of different types of vasopressors used during surgery also showed no significant effect on intraoperative or postoperative pedicle thrombosis or flap loss ($P = 0.162$).

CONCLUSIONS: Intraoperative use of vasopressors was common during free flap reconstruction. Use of phenylephrine, ephedrine, or calcium during any time of surgery did not increase the incidence of intraoperative or postoperative flap thrombosis or flap loss.

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Preoperative Versus Postoperative Language Specialization in Infants with Sagittal Craniosynostosis: An ERP Study

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PURPOSE: We compared perceptual narrowing, representative of language specialization, in infants with nonsyndromic sagittal craniosynostosis (NSC) preoperatively versus postoperatively using event-related potentials to measure impact of surgery on language processing. In refinement of our previous work, this assesses discrimination of native/nonnative speech instead of rudimentary response to general speech.

METHODS: Fifty infants (15 NSC and 35 control) completed an electroencephalogram (EEG) at age 3 to 6 months (presurgery); 33 infants (7 NSC and 26 control) completed a second EEG at age 12 to 14 months (postsurgery). During the EEG, infants listened to the dental /da/ and retroflex /da/ phonemes, present and not present in English, respectively. The mismatch negativity (MMN) event-related potential was extracted from EEG. Statistical analyses included *t* tests and repeated measures analysis of variance.

RESULTS: Preoperatively, there was a statistically significant ($P = 0.01$) difference in the MMN between the NSC versus control groups at the left frontal electrodes; the other regions showed no significant differences between groups ($P > 0.05$). Within groups, there was a significant effect of region within the left hemisphere of controls (frontal greater than central, $P = 0.009$), whereas in NSC, there was a significant effect of region within the right hemisphere (central greater than frontal, $P = 0.015$). Postoperatively, there was no significant difference in the MMN between the NSC versus control groups ($P > 0.5$) in all regions, and no significant within-group effects ($P > 0.2$) were observed.

CONCLUSIONS: There were significant differences in the MMN between NSC and control infants preoperatively but not postoperatively, which may suggest a more typical pattern of perceptual narrowing in NSC infants after surgery.

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Tafluprost, a Prostaglandin F2α Analog, Has Therapeutic Potential for Androgenic Alopecia through Modulation of Hair Cycle

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PURPOSE: Topical prostaglandin (PG) F2α was recently reported to promote hair growth, and a new PGF2α analog, tafluprost, was investigated in this study.

METHODS: Topical tafluprost was applied once daily on either telogen skin or depilation-induced anagen skin in C57BL/6 mice using 7 different concentrations, and hair growth was evaluated until day 42. The underlying mechanism was explored by various in vitro and in vivo approaches.

RESULTS: Tafluprost application on telogen skin promoted telogen-to-anagen conversion most at the same concentration as a commercial eye drop. Hair protrusion was observed much earlier (day 19.6±0.7) than control (day 42). In depilation-induced anagen mice, tafluprost elongated early anagen phase, but did not elongate mid-late anagen phase. In organ culture of human scalp hair and mice vibrissa follicles, hair growth was not accelerated by tafluprost, and proliferation of cultured human dermal papilla cells (hDPCs) was not affected by tafluprost in BrdU incorporation analysis. Chick chorioallantoic membrane assay showed no promotion of angiogenesis by tafluprost. Microarray using hDPCs and human keratinocytes (hKCs) showed that interleukin (IL)-1β, known to induce catagen, and inflammatory cytokines such as IL-6, IL-8, and CXCL2 were downregulated in tafluporst-treated hDPCs, whereas hair cycle-associated growth factors such as FGF-1, 2, and 7 were stable. Genes related to keratinization were upregulated in hKCs. The correlation between PGF2α, PGD2, and PGE2 was also evaluated in gene expression using real-time polymerase chain reaction, indicating the affected profile of these genes.

CONCLUSIONS: Tafluprost affected the function of hDPCs and hKCs and showed therapeutic potentials for androgenic alopecia by modulating hair cycle such as anagen induction and elongation of early anagen.

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Role of Hemangioma Stem Cell Notch3 in Infantile Hemangioma Blood Vessel Development

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PURPOSE: Infantile hemangiomas (IHs) are vascular tumors of infancy that occur within the first 5 years and proceed through a phase of rapid proliferation followed by involution. IHs are thought to originate from $CD133^+$ hemangioma stem cells (HemSCs). HemSCs are localized in perivascular regions of IH tissue and express NOTCH3, a protein involved in mural cell (vascular smooth muscle cell and pericyte) differentiation and maturation. We hypothesize that NOTCH3 functions in HemSCs to promote mural cell differentiation.

METHODS: Isolated $CD133^+$ HemSCs were infected with lentiviruses encoding a *NOTCH3* shRNA (N3KD) or scrambled control (SCR). To evaluate NOTCH3 function in HemSC differentiation in vitro, HemSCs were cultured with cord blood endothelial progenitor cells (cbEPCs), which express high levels of the NOTCH ligand, JAGGED1. To assess the role of HemSC in vivo, a mouse IH model was used in which HemSCs and cbEPCs were suspended in Matrigel and engrafted in immunodeficient mice. IH matrigel implants were harvested for histological analysis after 14 days.

RESULTS: HemSCs differentiated into α-SMA+ cells when cocultured with cbEPCs in vitro. In N3KD HemSCs, α-SMA expression was reduced when compared with control HemSCs. In vivo, Doppler ultrasound revealed vascular structures with blood flow in control HemSC implants. Blood flow was reduced in N3KD HemSCs implants. Histological analysis of control implants revealed ectatic vessels with typical IH morphology supported by α-SMA+ mural cells. In contrast, IH matrigel implants with N3KD HemSCs had decreased α-SMA+ mural cell coverage and poor vessel integrity.

CONCLUSIONS: In IHs, NOTCH3 promotes HemSC differentiation into α-SMA+ perivascular cells that may support pathological IH blood vessel stability, suggesting that NOTCH3 is a potential therapeutic target.

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Targeted Topical Antioxidant Therapy in Diabetic Wound Healing

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PURPOSE: Currently, no effective pharmacological agents exist for the treatment of chronic nonhealing diabetic wounds. In previous study, we found dysfunctional Nrf2-Keap1 signaling in diabetic skin but restored the pathway to enhance wound healing. In this study, we assessed the efficacy of an Nrf2 activator (NA), hypothesizing that topical application accelerates diabetic wound healing.

METHODS: We topically administered high-dose (HD)-NA, lose-dose (LD)-NA, or vehicle daily to 10-mm diameter excisional humanized diabetic cutaneous wounds. We assessed wound time to closure, in vivo real-time ROS, and 10-day-old wounds for histological and molecular analysis.

RESULTS: LD-NA reduced healing time to 21.6 versus 30 days in vehicle-treated wounds, with highest wound closure rate versus vehicle, $P = 0.0007$. LD decreased pathologic healing time by 52% versus untreated wounds and by 41% versus vehicle. LD decreases wound burden 65% versus untreated, 48% versus vehicle, $P < 0.05$. LD induces 72% ROS reduction in vivo, versus vehicle, $P < 0.01$. HD does not significantly alter wound healing. Histologically, 10-day-old LD wounds showed least epithelial gap, 250% granulation tissue, and 200% $CD31^+$ vasculature, versus vehicle, all $P < 0.01$. We found nuclear Nrf2 with NA, unlike vehicle. Both LD and HD upregulate Nrf2 target gene *NQO1* by 6.5- and 10-fold, respectively, but do not affect MnSOD.

CONCLUSIONS: Topical LD-NA significantly decreases diabetic cutaneous wound healing time by altering the redox status of the wound bed through Nrf2-transcribed antioxidant genes. We demonstrate an effective strategy to treat chronic diabetic wounds and support the development of NA for clinical application.

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HIF-2α Critically Regulates Metabolism and Homeostasis in Long Bones

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PURPOSE: Hypoxia inducible factor-1α (HIF-1α) plays a critical role in angiogenesis and regulation of osteoblast metabolism. Similarly, HIF-2α is expressed in cells of the osteoblast lineage; however, its exact role in bone formation and homeostasis remains to be determined.

METHODS: We generated a novel gain-of-function mouse model in which PRX-1Cre transgenic mice were crossed to HIF-2α dPAf/f. In vivo analysis was conducted at 6 and 12 weeks of age, respectively, by nano computed tomography, routine histology, and histomorphometry. Moreover, bone marrow stromal cells overexpressing HIF-2α were subjected to microarray analysis.

RESULTS: HIF-2a overexpressing mice demonstrated distinct morphologic changes in long bone size and shape. Although trabecular density was increased in mutant mice on nano computed tomography, this was highly disorganized with thinner trabeculae compared with controls. Furthermore, increased number of cartilage remnants was observed in mutant mice suggesting elevated resorption because of altered osteoclast function. This may be contributed by upregulation of lysyl oxidase and downregulation of metalloproteinase 13 in mutant cells as seen on microarray analysis.

CONCLUSIONS: We demonstrate that overexpression of HIF-2α affects the overall size, shape, and composition of long bones. Moreover, HIF-2α critically modulates bone metabolism and may alter the quality of matrix deposited by osteoblasts. These findings help define the role of HIF-2α in osteogenesis and may enable strategies in the treatment of patients with disorders of bone development.

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Efficient Assessment of Lymphangiogenesis Using a Lymphatic Reporter Mouse and Its Embryonic Stem Cells

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PURPOSE: Lymphatic reporter mouse lines are important tools in the study of the lymphatic vascular system. Unfortunately, many are limited by their reliance on promoters that fail to recapitulate endogenous gene expression patterns. In this study, we aimed to evaluate the reporter expression pattern of the novel lymphatic reporter line Prox1-tdTomato and to assess its value in studying lymphangiogenesis.

METHODS: Reporter expression pattern was evaluated using fluorescent imaging in embryos and adult mouse tissue. Lymphangiogenesis was evaluated using unilateral axillary lymphadenectomy, axillary lymph node transplantation, and a mouse tail lymphedema model. Embryonic stem cells (ESCs) from the reporter line were implanted in NOD-SCID mice to examine their ability to differentiate into lymphatic endothelium.

RESULTS: Strong reporter expression consistent with endogenous Prox1 expression enabled direct visualization of well-organized lymphatic vascular networks in the expected distribution of tissues, including skin, mesentery, and lymph nodes. This permitted rapid characterization of lymphangiogenesis in all models. Reporter line ESCs implanted into NOD-SCID mice differentiated into lymphatic endothelium and formed structurally normal lymphatic vessels.

CONCLUSIONS: The Prox1-tdTomato reporter line recapitulates endogenous lymphatic-specific gene expression patterns and enables the direct visualization of lymphangiogenesis in a variety of models. Reporter line ESCs may be useful for the real-time monitoring of lymphatic endothelial differentiation, elucidating an otherwise poorly understood process.

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Antiproliferative Effect of Propranolol on Lymphatic Malformations

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PURPOSE: Lymphatic malformations (LMs) are congenital vascular lesions with severe morbidities and a high recurrence rate (up to 57%). Propranolol, a β-adrenergic receptor (βAR) antagonist, is effective in a subset of LM patients. We find that β1AR and β2AR expression is increased in lymphatic endothelial cells (LECs) in LM tissues relative to control tissues, whereas propranolol inhibits proliferation of LM-derived LECs. We hypothesized that LECs in LMs have increased proliferation that is targeted by propranolol.

METHODS: LM tissues (n = 9) or neonatal foreskins (control; n = 3) were costained for podoplanin (LEC marker) and Ki67 (proliferative marker). To assess propranolol effects on LEC gene expression, LM-derived LECs were treated with propranolol or vehicle for 48 hours and quantitative reverse transcription polymerase chain reaction performed for *LEC* genes. To assess in vivo, LM cells in Matrigel were implanted in nude mice; half were treated with propranolol or vehicle (n = 4) for 5 weeks. Sections were stained for podoplanin, and lymphatic phenotype was assessed. Significance was determined by two-tailed Student's *t* test.

RESULTS: LM tissues displayed increased LEC proliferation associated with abnormal lumen dilation relative to controls. Propranolol increased expression of LEC proteins, podoplanin, LYVE1, VEGFR-2, and VEGFR-3. In the in vivo model, propranolol reduced lymphatic vessel density and dilation.

CONCLUSIONS: We demonstrate that LMs, which have been considered quiescent lesions, have increased LEC proliferation that may contribute to abnormal vessel dilation and dysfunction. In LM patients, propranolol may reduce abnormal LEC proliferation and increase LEC differentiation to normalize lymphatic vessel phenotype.

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Intraperitoneally Administered PEGylated NELL-1 Treatment for Osteoporosis, in Mice

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PURPOSE: Osteoporosis affects more than 200 million people worldwide. Current antiresorptive treatments have significant limitations because of systemic side effects. NELL-1 is a potent cytokine with combined proosteogenic and antiosteoclastic effects. Previous systemic administration studies using PEGylated NELL-1(NELL-PEG) showed increased bone quality and quantity in healthy mice given 1.25 mg/kg intravenously every 7 days. This study aims to reduce NELL-PEG injection frequency to 14 days while using more patient-friendly intraperitoneal (IP) administration route to recover bone in OVX-induced osteoporotic mice.

METHODS: Three-month-old female BALB/c mice underwent ovariectomy (OVX; n = 10) or Sham operation (n = 10). Osteoporosis induction was confirmed using dual-energy x-ray absorptiometry and ex vivo micro computed tomography (CT) scans. Twelve 5-week-post-OVX BALB/c mice underwent NELL-PEG treatment every 2 weeks with dosages of (1) 5 mg/kg IP or (2) 10 mg/kg IP and harvested at 8 weeks. Serum was analyzed at 0, 4, and 8 weeks. Dynamic BMD was measured by dual-energy x-ray absorptiometry. Ex vivo micro CT, colony-forming unit assay, and histology were performed after harvest.

RESULTS: Ovariectomy was confirmed by decreased BMD (7%) and BV/TV (39.3%). Systemic NELL-PEG at 10 mg/kg IP increased BMD in distal femur (13%), proximal tibia (14%), and lumbar vertebrae (15%). Micro CT data showed the same trend in BMD, BV/TV, and trabecular structures. Colony-forming unit assay confirmed greater osteogenesis in bone marrow stem cells flushed from 10 mg/kg delivery than 5 mg/kg group, and histology confirmed increased bone formation in both treatment groups.

CONCLUSIONS: Systemic NELL-PEG therapy regenerates bone in osteoporotic mice when administered at 10 mg/kg dosage via IP administration every 2 weeks. Systemic NELL-PEG may be applied to improve overall bone architecture and potentially enhance postsurgical craniofacial and alveolar bone healing.

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Reduction of Accumulated Reactive Oxygen Species Can Be Achieved By Bathing Standard Lipoaspirate in Oxygenated Micro/Nanobubbles

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PURPOSE: Micro/nanobubbles (MNBs) are gaining attention in the field of medicine because of their stability in solution and potential as a therapeutic delivery system. Here, we tested whether oxygenated MNBs could be used to reduce the effects of hypoxia on standard liposuction aspirate.

METHODS: Lipoaspirate from a routine liposuction case was maintained at room temperature for 24 hours. Oxygenated MNBs were infused into phosphate-buffered saline (PBS) to a concentration >20 mg/L using our custom MNB generator. The lipoaspirate was bathed in either PBS or PBS + MNBs for 15, 30, or 60 minutes on a rocker. At each time point, samples were snap frozen in liquid nitrogen, cut to a thickness of 25 μm, and stained with dihydroethidium (DHE) and 4′,6-diamidino-2-phenylindole (DAPI). Images were obtained using a fluorescence microscope. Positive nuclear staining (DAPI) versus reactive oxygen species (ROS) staining (DHE) were quantified and represented as a ratio (DAPI/DHE).

RESULTS: Standard lipoaspirate that was bathed in oxygenated MNBs expressed a significant reduction in accumulated ROS at 15 and 30 minutes when compared with the control group (1.01 ± 0.04 vs 0.85 ± 0.13, $P = 0.05$ and 1.27 ± 0.22 vs 1.10 ± 0.14, $P = 0.05$, respectively). There was no comparable reduction in ROS for either group at 1 hour.

CONCLUSIONS: Buffered saline oxygenated with MNBs can be used as a bathing solution for lipoaspirate to mitigate the deleterious effects of hypoxia including the generation of ROS. Further studies are needed to determine the clinical significance of these findings.

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A Novel Experimental Rat Skin Flap Model that Distinguish between Venous Congestion and Arterial Ischemia: The Reverse U-Shaped Bipedicled Superficial Inferior Epigastric Artery Flap

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PURPOSE: A rat skin flap model has been used in numerous studies with evaluation of the flap survival rate. Flap necrosis is caused by mixed arterial and venous insufficiency. However, there is no flap model that can clearly distinguish between ischemia and congestion.

METHODS: We created a new rat skin flap model to distinguish between venous congestion and arterial ischemia. Rats were divided into 3 groups: control (n = 3), ischemia (n = 10), and congestion (n = 10). A reverse U-shaped bipedicled superficial epigastric artery flap was elevated. On the opposite side to the pedicle, the artery was ligated as an ischemia model (A-V+), and vein was ligated as a congestion model (A+V−). The flap was returned to the original site and sutured. Surrounding neovascularization was blocked by polyurethane film. The flap survival rate was evaluated on the third postoperative day, and statistical analysis (1-way analysis of variance) was performed.

RESULTS: The mean flap survival rate was 100%, 61.8% (56.9–67.1%), and 42.3% (35.7%–48.7%) in the control, ischemia, and congestion groups, respectively ($P < 0.001$). Our results demonstrated that the present flap could significantly distinguish between ischemia and congestion.

CONCLUSIONS: This flap model is simple and has a consistent flap survival rate. We believe that this flap model can be used to assess the benefits of various pharmacological agents on the survival pattern of skin flaps.

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Mrp-1-Dependent Gssg Efflux as a Critical Survival Factor for Oxidant Enriched Tumor Forming Endothelial Cells

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PURPOSE: Highly elevated levels of Nox-4, a protein that generates H_2O_2, is seen in both human hemangiomas and a murine model of hemangioendothelioma. Tumor-forming endothelial (EOMA) cells are able to escape cell death despite excessive Nox-4-derived nuclear oxidant burden. The objective of this study was to characterize the mechanisms by which EOMA cells evade oxidant toxicity and thrive.

METHODS: Multidrug resistance-associated protein-1 (MRP-1) is a transport protein that eliminates toxic molecules from cells including chemotherapy agents such as vinca alkaloids and oxidized glutathione (GSSG). MRP-1 activity was measured by calcein exclusion and glutathione measurements were taken using high-performance liquid chromatography.

RESULTS: In EOMA, nuclear GSSG/GSH ratio was 5-fold higher compared with cytosol. Compared with those in healthy murine arterial endothelial cells (MAEs), cellular GSSG/GSH was over twice in EOMA. MRP-1 activity was twice as high in EOMA compared with MAE. Hyperactive YB-1 and Ape/Ref-1 were responsible for high MRP-1 inhibition, and knockdown resulted in elevation of nuclear GSSG causing death of EOMA cells. Disulfide loading of cells by inhibition of GSSG reductase was effective in causing EOMA death as well. In summary, EOMA cells survive a heavy oxidant burden by rapid efflux of GSSG, which is lethal if trapped within the cell.

CONCLUSIONS: A hyperactive MRP-1 system for GSSG efflux acts as a critical survival factor for these cells, but it also promotes chemotherapy resistance. MRP-1 may be a productive target for endothelial cell tumor therapeutics.

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Stimulation of Adipose-Derived Stem Cells with Interferon Gamma Induces Trail-Mediated Apoptosis in Triple Negative Breast Cancer

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PURPOSE: Stem cell-based reconstructive techniques offer minimally invasive options for autologous breast reconstruction, but there are concerns regarding their oncological safety in cancer patients. In this study, we investigate whether pretreatment of human adipose-derived stem cells (ASCs) could induce apoptosis in breast cancer cells and therefore confer oncological benefits in reconstruction.

METHODS: ASCs were isolated from human abdominal fat and expanded in vitro. ASCs were exposed to interferon (IFN)-γ in culture for 72 hours. The effect of IFN-γ exposure on TRAIL expression was determined. The mesenchymal triple negative breast cancer cell line MDA-MB-231 was cocultured with IFN-γ-treated ASCs for 72 hours. The effect on MDA-MB-231 cell survival was determined.

RESULTS: Untreated ASCs did not express TRAIL. Exposure to IFN-γ induced TRAIL expression in ASCs in a dose-dependent manner. Coculture of IFN-γ-treated ASCs with the MDA-MB-231 cell line resulted in significant apoptosis of cancer cells. Untreated ASCs did not significantly alter MDA-MB-231 proliferation rates. The presence of TRAIL death receptors (DR1/DR2) on MDA-MB-231 breast cancer cells was confirmed. Inhibition of TRAIL reversed the apoptotic effect of IFN-γ-treated ASCs. Upregulation of caspase 3/7 confirmed apoptotic cell death

CONCLUSIONS: Pretreatment of ASCs with IFN-γ induces TRAIL expression resulting in apoptosis of the mesenchymal triple negative MDA-MB-231 breast cancer cell line. IFN-γ treatment of ASCs may confer oncological benefits and improve the safety of stem cell-based reconstructive strategies in patients with TRAIL sensitive tumor types.

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Macrophage-Mediated Production of BMP Ligand Induces Tendon Degeneration and Heterotopic Ossification

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PURPOSE: Heterotopic ossification (HO) after trauma is characterized by upregulation of transforming growth factor (TGF)-β/Smad signaling. Bone morphogenetic proteins (BMPs) act as a major intermediary along this pathway. One of the clinical hallmarks of HO-causing injuries severe and sustained inflammation. Here, we redemonstrate the importance of TGF-β/Smad signaling in the formation of HO and identify macrophages as a possible mediator of this signal posttraumatic HO.

METHODS: C57Bl/6 and tamoxifen-inducible BMP receptor knockout mice (*Ub.creERT;Acvr1*$^{fl/fl}$) underwent Achilles' tenotomy and 30% TBSA burn. Mice were survived up to 9 weeks postinjury for micro computed tomography analysis. Separate burn/tenotomy mice were killed 48 hours and 1 week postinjury to isolate macrophages during the earliest stages of HO development. Flow cytometry was used to analyze F4/80+ macrophages for expression of TGF-β and BMP2. Finally, burn/tenotomy mice were depleted of macrophages with clodronate and killed at 3 weeks to evaluate cartilage formation.

RESULTS: BMP receptor knockout mice (*Ub.creERT;Acvr1*$^{fl/fl}$) produced significantly less HO versus littermate controls (A). F4/80+ macrophages were present by 48 hours and comprised 14.93% ± 3.49% of the cells at the tenotomy site by 1 week postinjury. BMP2 was expressed by 40.10% ± 5.26% of macrophages and TGF-β by 93.87% ± 2.19% (B). Depletion of macrophages using clodronate reduced cartilage presence.

CONCLUSIONS: Cartilage formation in HO is preceded by infiltration of macrophages, which express prochondrogenic factors including TGF-β and BMP2. Macrophage depletion reduces cartilage formation, suggesting a mechanistic role for these cells.

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Keratinocyte Migration Is Affected by Fibroblast Subtype

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PURPOSE: Chronic wounds affect 6 million people in the United States alone and are challenging to treat, leading to prolonged debility. Wound healing is a complex process requiring an inflammatory response, fibroblast proliferation, matrix production, and keratinocyte migration to close the wound. Although keratinocytes at the edge of a chronic wound are highly proliferative, they fail to migrate. We hypothesize that papillary fibroblasts present at the exposed surface of a superficial, easily healable, wound stimulate keratinocyte migration more effectively than reticular fibroblasts present at the exposed surface of a deep or chronic wound. Identification of factors that enhance keratinocyte migration could provide new treatment for chronic wounds.

METHODS: Site-matched human keratinocytes and dermal fibroblasts were isolated from discarded skin during surgery. Papillary and reticular fibroblasts were cultured separately. Keratinocytes were grown on an insert, and migration was assessed using a standard scratch assay with keratinocytes exposed to media, papillary, or reticular fibroblasts.

RESULTS: Fibroblast type dramatically affected epithelial migration. Keratinocytes exposed to papillary fibroblasts exhibited a 58% closure of the scratch over 8 hours, whereas that exposed to reticular fibroblasts stimulated a 73% reduction (P = 0.02). When exposed to mixed cultures containing both fibroblast subtypes, keratinocytes closed the scratch by 88%. Keratinocytes subjected to media alone demonstrated a basal migration of 36%.

CONCLUSIONS: These data provide strong evidence for how cells interact in the wound environment to optimize healing. Studies are ongoing to identify secreted factors, which mediate enhanced keratinocyte migration. Future experiments will consider dysfunctional support of epithelial migration by chronic wound fibroblasts.

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Obesity-Induced Perilymphatic Inflammation Impairs Lymphatic Function

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PURPOSE: Diet-induced obesity is a known risk factor for lymphedema. However, although it is clear that obesity is associated with lymphatic dysfunction, it remains unclear whether this is because of increased subcutaneous adipose deposition or dietary toxins. Therefore, the purpose of this study was to evaluate the independent effects of high-fat diet or obesity on lymphatic function in obesity-prone and obesity-resistant mice.

METHODS: Male C57BL/6J (obesity prone) and MSTNln or Balb/cJ (obesity resistant) mice were maintained on a normal chow or high-fat diet for 10 weeks followed by analysis of lymphatic function. Correlative in vitro studies were performed to analyze VEGFR-3 signaling pathways in isolated lymphatic endothelial cells (LECs).

RESULTS: Only obese mice developed leaky initial lymphatics, decreased lymphatic antigen and colloid transport function, and increased perilymphatic inflammation ($P < 0.001$), suggesting that subcutaneous adipose deposition is necessary for this response. These findings correlated with a marked decrease in LECs gene expression of lymphatic differentiation genes *VEGFR-3* and *Prox1*. In contrast, LECs from obesity-resistant mice had normal expression patterns compared with their NCD controls. In vitro exposure of LECs to free fatty acid resulted in decreased *VEGFR-3* expression and a dose-response decrease in cell viability. These effects were abrogated by increasing VEGFR-3 signaling.

CONCLUSIONS: Our findings suggest that obesity-mediated lymphatic injury is because of increased subcutaneous adipose deposition and low-grade perilymphatic inflammation rather than direct harmful effects of dietary toxins. This lymphatic injury correlates with decreased expression of *VEGFR-3* and *Prox1*. Increasing VEGFR-3 signaling confers significant protection to LECs and decreases lymphatic injury.

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A Novel, Transgenic, Inducible Animal Model of Scrotal Lymphedema

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PURPOSE: Scrotal lymphedema is a challenging problem and affects nearly 30 million men worldwide. Treatments are radical and deforming, and development of therapies has been limited by a lack of adequate animal models. To address this gap, we developed a novel transgenic animal model of scrotal lymphedema by ablating the lymphatic system with diphtheria toxin.

METHODS: We created Cre-lox mice that express the human diphtheria toxin receptor selectively on lymphatic endothelial cells using a lymphatic specific promoter (FLT-4). This receptor binds diphtheria toxin avidly and results in selective ablation of the target cells. We then injected diphtheria toxin in the scrotum to selectively ablate the lymphatic vessels in this region and analyzed the development of scrotal lymphedema as well as its pathological changes over time.

RESULTS: One week after ablation, scrotal volumes increased to 520% of normal, and this volume increase was sustained for at least 4 weeks. We noted a 66% reduction in dermal capillary lymphatic vessel density ($P = 0.003$) and an 88% reduction in collecting lymphatic vessels ($P < 0.001$). There was a 70% increase in T-cell soft tissue infiltration ($P = 0.038$), but no difference in macrophages. There was also an 80% increase in epidermal thickness ($P < 0.001$). Residual lymphatics were highly abnormal and had significant accumulation of perilymphatic inflammatory cells.

CONCLUSIONS: We have created and described the first animal model of scrotal lymphedema. Further experiments with this model may allow for new discoveries of scrotal lymphatic function/dysfunction and will allow for the testing of surgical and pharmacological treatment possibilities.

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Vascularized Composite Allograft Tolerance with Costimulatory Blockade and Transient Tacrolimus in a Large Animal Model

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PURPOSE: Costimulatory blockade with belatacept, a cytotoxic T lymphocyte-associated antigen-4 immunoglobulin, has shown promise in solid organ transplantation. The aim of this study was to investigate the efficacy of belatacept to reduce the requirement for conventional immunosuppression such as calcineurin inhibitors in maintaining vascularized composite allograft (VCA) survival in a large animal model.

METHODS: Heterotopic osteomyocutaneous hindlimb transplantation was performed in 16 MGH miniature swine across full swine leukocyte antigen mismatch. All animals received nonmyeloablative conditioning with 50 cGy total body and 300 cGy thymic irradiation pretransplant.

RESULTS: High-dose tacrolimus led to maintenance of VCA in 3 of 3 animals but was associated with major infectious complications. Two of 3 animals in group II rejected their graft by postoperative day (POD) 46 and POD217. In group III, 2 of 5 animals demonstrated rejection before POD150, whereas 3 of 5 animals achieved tolerance of their VCA with graft survival beyond POD300. Five of 5 animals in group IV has maintained graft survival beyond POD170 without rejection.

CONCLUSIONS: Previously, VCA tolerance in large animal models have required establishment of chimerism by either donor bone marrow infusion or hematopoietic stem cell transplantation. The results of this study suggest that tolerance of VCA containing vascularized bone marrow can be achieved with our conditioning regimen of belatacept and peritransplant tacrolimus without the requirement for myeloablative conditioning.

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Oxygen Tension and Indolamine 2, 3-Dioxygenase Expression Regulate Mesenchymal Stem Cell–Mediated Regulatory T-Cell Expansion

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PURPOSE: Mesenchymal stem cells (MSCs) have powerful immunosuppressive properties, partially mediated by expansion of regulatory T cells (Tregs) populations. We previously showed that priming MSCs with hypoxic and inflammatory conditions enhances their stem-like and immunomodulatory properties, largely through induction of indolamine 2, 3-dioxygenase (IDO). We, therefore, hypothesize that priming MSCs will increase MSC-mediated Treg expansion through an IDO-specific mechanism.

METHODS: We harvested $CD4^+$ T cells from Lewis rat spleens and cocultured them with MSCs and allogeneic rat endothelial cells (ECs). MSCs were cultured in either hypoxia (5% O_2) or normoxia, or primed with inflammatory cytokine interferon-γ. Flow cytometry for FoxP3 determined %Tregs.

RESULTS: With MSC/$CD4^+$/EC coculture, %Tregs tripled to 24.13% ± 2.16%, versus $CD4^+$/EC(9.28% ± 1.15%) or $CD4^+$ alone (7.94% ± 1.22%), $P < 0.0001$ for both. When MSCs were physically separated in culture from $CD4^+$/ECs, %Tregs was similar to MSC/$CD4^+$/EC (27.77% ± 1.99% vs 22.6% ± 5.65%). By using hypoxia-primed MSCs, %Tregs more than doubled to 24.13% ± 2.16% versus 11.06% ± 2.19% with normoxic MSCs ($P < 0.01$). When we inhibited IDO, %Tregs significantly decreased to 12.4% ± 3.10% ($P < 0.01$), almost negating effects of MSCs on Treg proliferation. Priming MSCs with IFN-γ had no impact on %Tregs in normoxia and hypoxia.

CONCLUSIONS: Addition of autologous MSCs to coculture increases Treg proliferation, which is potentiated by hypoxia-primed MSCs. MSC-mediated Treg expansion does not require direct contact, indicating a paracrine mechanism. Inhibition of IDO significantly decreases the proliferation of Tregs, implying a key role of IDO. These results further delineate the mechanisms by which MSCs exert their immunomodulatory functions, demonstrating methods to prime MSCs to optimize their immunomodulation.

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Nonmyeloablative Immunosuppression Prevents Rejection of Vascularized Composite Allografts

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PURPOSE: Reconstructive transplantation represents a valid therapeutic option after devastating tissue loss. Routine clinical application of this approach, however, is hampered by the toxicity of long-term maintenance immunosuppression. This study investigated a novel short-term immunomodulatory strategy in a murine hindlimb transplantation model.

METHODS: Fully MHC-mismatched orthotopic hindlimb transplants were performed from Balb/C to C57BL/6 mice. Recipient animals received combinations of T-depleting monoclonal antibody, cytotoxic T lymphocyte-associated antigen-4 immunoglobulin, and rapamycin [postoperative day (POD) 0–9]. Flow cytometric analysis was performed to evaluate mixed chimerism and clonal deletion of alloreactive T cells.

RESULTS: CTLA4-Ig-treated group showed increased graft survival compared with the nontreated controls. Combination of T-cell depletion and cytotoxic T lymphocyte-associated antigen-4 immunoglobulin plus short course of rapamycin increased vascularized composite allograft survival significantly (MST, 105 days; $P < 0.01$). Mixed chimerism was detected in recipients receiving the combined treatment protocol with 5.013% ± 1.23 % of donor-derived $CD11b^+$ cells on POD55. Vβ−TCR staining profiles in recipients after full treatment showed 1.570% ± 0.3700% of νβ5+$CD4^+$ T cells, whereas naive C57BL/6 express 3.567% ± 0.3690% of νβ5+$CD4^+$ T cells, suggesting the actuation of central deletion of developing donor-reactive T cells. Graft-infiltrating Foxp3+ regulatory T cells of donor origin were detected on POD60, suggesting an important regulatory role exerted by donor regulatory T cell.

CONCLUSIONS: This study shows that the combination of T cell depletion, costimulation blockade, and a short course of rapamycin prevents vascularized composite allograft rejection and significantly prolongs graft survival without myeloablative conditioning. Immunoregulation appears to rely on the combined effect of mixed chimerism and regulatory T cells.

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A Novel Rat Forelimb Transplantation Model that Allows for Optimized Measurement of Functional Recovery after VCA

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PURPOSE: Studies to investigate strategies to improve functional outcomes in VCA are limited by the lack of appropriate animal models. The rat hindlimb transplant model is used for histologic and electrophysiological measures of nerve regeneration but does not allow for reliable assessment of behavioral functional recovery. We developed a novel forelimb transplant model to address this specific problem.

METHODS: Rat orthotopic forelimb transplantation was performed at the mid-humerus level with anastomosis of brachial vessels. Median and radial nerves were approximated in the experimental group, but left in discontinuity in the control group (N = 6/group) to determine the degree of functional return because of nerve regeneration. Functional recovery was tested by measuring grip strength using a force transducer and evaluating performance of repetitive feeding movements. Median nerve histomorphometry and flexor digitorum myofiber cross-sectional area analysis were performed at 12 weeks.

RESULTS: Long-term allograft survival (>120 days) was successfully achieved with cyclosporine A (10 mg/kg/day). Animals in the control group did not regain grip strength after transplantation, whereas the experimental group demonstrated 57% ± 5.5% return of baseline grip strength ($P < 0.05$). Forelimb function scores (0–9) were significantly greater in the experimental group than the controls (3.7 ± 0.49 vs 1.2 ± 0.37; $P < 0.05$). Muscle histology and nerve histomorphometry are pending.

CONCLUSIONS: Rat forelimb transplantation represents the first VCA model that allows for reliable and reproducible measurement of functional recovery in addition to histologic measures of reinnervation.

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Exosomes from Mesenchymal Stem Cells Are Antiinflammatory Mediators in VCA

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PURPOSE: Mesenchymal stem cells (MSCs) are immunomodulators investigated in vascularized composite allotransplantation (VCA), yet their mechanism is poorly defined. We hypothesized that MSCs package antiinflammatory cytokines into exosomes (EXOs), which execute the immunomodulatory effects of MSCs, and differential EXO packaging could be driven by oxygen tension and inflammatory milieu. Our aims were to (1) determine whether MSCs secrete EXOs; (2) characterize MSC-EXO cargo under differential conditions; (3) investigate MSC-EXOs in preventing rejection in VCA.

METHODS: Lewis rat-derived MSCs were cultured in normoxia (21% O_2), hypoxia (5% O_2), or with 100 U interferon (IFN)-γ. MSC-EXOs were isolated by ultracentrifugation and confirmed by TEM, immunoblot, and flow cytometry for EXO markers. MSC-EXO cytokine cargo were characterized by flow cytometry and assessed for syngeneic regulatory T cell (Treg) induction and in an allogeneic rat hindlimb VCA model.

RESULTS: MSC-EXOs from normoxic, hypoxic, and IFN-γ-primed MSCs expressed EXO markers Cd63 and Cd81. Hypoxic MSC-EXOs packaged significantly greater quantities of indolamine 2, 3-dioxygenase (6.4-fold, $P < 0.0001$), interleukin-10 (1.4-fold, $P < 0.05$), inducible nitric oxide synthase (2.1-fold, $P < 0.0001$), PGE2 (1.7-fold, $P < 0.05$), and transforming growth factor-β (1.4-fold, $P < 0.005$) versus an isotype control. IFN-γ-primed MSCs packaged greater quantities of inducible nitric oxide synthase and transforming growth factor-β compared with normoxic and hypoxic MSC-EXOs ($P < 0.05$ for all). Normoxic MSC-EXOs induced a 6-fold increase in a $CD4^+$FoxP3+ Treg population ($P < 0.005$) versus negative control; a 2-fold greater induction than normoxic MSCs ($P < 0.0001$). In a VCA model, ex vivo seeded DiI-labeled MSC-EXOs localized to the perivascular space after reimplantation.

CONCLUSIONS: MSC-EXOs package all known mediators of MSC immunomodulation and stimulate Treg production. Preliminary VCA studies suggest that MSC-EXOs localize to sites of immunologic importance similar to MSCs. MSC-EXOs are a promising explanation for MSC immunomodulatory properties and may offer a novel therapeutic approach in VCA.

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Challenges of Triple Immunosuppression in Nonhuman Primate Models of Vascularized Composite Allotransplantation

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PURPOSE: Tolerance of renal and lung allografts in nonhuman primates (NHPs) has been achieved by bone marrow transplantation 4 months after solid organ transplant and recipients receive triple immunosuppression (FK506, MMF, and methylprednisolone) in the interim. This delayed tolerance induction protocol has been adopted in NHP VCA studies. We report the challenges of triple immunosuppression during the 4-month delay.

METHODS: To prevent acute rejection, NHPs received induction therapy (antithymocyte globulin) pre-VCA. Post-VCA, NHPs were maintained on triple immunosuppression and followed closely with serial biopsies (of VCA and host skin) and immunologic assays; further biopsies were performed in suspected acute rejection.

RESULTS: Post-VCA, acute rejection (1–3 episodes, between 1 and 3 months) and other complications coincided with the near complete turnover of skin resident leukocytes (by flow cytometric analysis) in the VCA to recipient-origin cells by postoperative day 30. In all recipients, there was no evidence of donor-specific antibodies or in vitro donor responses.

CONCLUSIONS: Unlike solid organ transplant, triple immunosuppression may not be sufficient for VCA during the original 4-month delay in the delayed tolerance induction protocol. Acute rejection likely developed when immunosuppression levels fluctuated; the overall immunosuppressive load led to systemic complications necessitating euthanasia. Further studies with a shortened delay period and overall duration of immunosuppression are underway for earlier bone marrow transplantation and tolerance induction.

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AMD3100 (Plerixafor) as a Single-Dose Stem Cell Mobilizing Agent in Vascularized Composite Tissue Allograft Transplantation in a Canine Haploidentical Model

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PURPOSE: Vascularized composite allograft (VCA) transplantation is a clinical reality but limited by toxicities of chronic immunosuppression and rejection. Current clinical tolerance protocols rely on recipient conditioning and donor cell mobilization limiting use to living donor transplants. We sought to design a clinically relevant protocol applicable to cadaveric organs. We modified our existing nonmyeloablative stem cell canine VCA transplant model to use AMD3100 (plerixafor) for stem cell mobilization.

METHODS: Five DLA-haploidentical, related canine recipients received conditioning with 350 cGy TBI, AMD3100-mobilized donor stem cells and VCA transplantation with a short course of immunosuppression (MMF: 84 days/CSP: 133 days; including taper). $CD34^+$ hematopoietic progenitor cells were quantified through flow cytometry. Peripheral blood chimerism was evaluated by polymerase chain reaction techniques weekly. VCA graft survival was followed up clinically and histologically.

RESULTS: All 5 canines tolerated the conditioning regimen. Four were followed up long term. Stem cell engraftment and donor chimerism were seen in all dogs. Median COBE apheresis cell counts of 6.12×10 cells/kg and $CD34^+$ cell count of 5.27×10^7 cells/kg were obtained. No acute rejection nor evidence of graft-versus-host disease was seen. An unexpected finding of persistent thrombocytopenia resolved on loss of donor cell chimerism.

CONCLUSIONS: This study demonstrates proof of principle for AMD3100 as a single-dose stem cell mobilizing agent for a clinically relevant tolerance protocol. The use of AMD3100 led to stem cell engraftment in all animals transplanted with no evidence of acute rejection in the VCA. Current application of AMD3100 is limited by thrombocytopenia but we are currently modifying the protocol to address this issue.

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Software-Based Video Analysis of Functional Outcomes of Face Transplantation

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PURPOSE: Outcome assessment of face transplantation is mandatory to provide evidence about this live-giving surgery. Current methods, however, are imprecise or prone to subjectivity. Software-based video analysis allows for fast, objective, and retrospective assessment of facial movements and expression of emotions.

METHODS: We recorded videos of each of our 7 face transplant recipients before and every 3 to 6 months after transplantation. In every video, each patient performs the same sequence of facial movements. These videos were retrospectively analyzed by a customized software, which is capable for automatic tracking and detailed measurements of facial movements and expressions. Subsequently, measurements were compared with the same patient at different time points, with unaffected structures in case of partial face transplants, and with a normal population.

RESULTS: After a mean follow-up of 3.5 years, every patient showed a significant improvement in facial movements compared with pretransplant time points. Significant improvements were reached after an average of 1.5 years after transplantation. In 3 patients with partial face transplants, extent and quality of facial movements of the transplanted part did not show any significant differences compared with the unaffected part 1 year after transplantation. Two partial and 1 full face transplant achieved outcomes comparable with a normal population.

CONCLUSIONS: Software-based video analysis provides the first assessment tool capable for objective, precise, and reproducible analysis of facial movements and emotions. Of note, this software works with conventional camera and computer equipment making it worldwide applicable

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Establishing a Novel Surgical Model for Optic Nerve Repair

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PURPOSE: Efforts to transplant a mammalian eye have failed mainly because of the ganglion cell axon's inability to withstand transection. Eye transplantation is not feasible without proof of optic nerve regeneration. The objective of this pilot study is to find an optimal rat optic nerve transection and repair model in preparation for eye transplantation.

METHODS: Thirty cadaver and 10 live Lewis rats were used to expose, transect, and repair the optic nerve and visualize its anatomical relationship with the ophthalmic artery. Nerve coaptation was performed with 12/0 suture. The pathway of the ophthalmic artery was evaluated by red latex injection.

RESULTS: A combined superior orbital rim and lateral canthotomy with partial temporalis muscle excision approach provided ideal access and sufficient operative space. The extraocular muscles were mobilized, and the Harderian and lacrimal glands were retracted without gland excision. The ophthalmic artery branches approximately 1.01 to 3.8 mm proximal to the fundus. The retinal artery crosses the optic nerve postero-nasal pathway and divides the smaller branches 0.81 to 1.12 mm proximal to the globe. A superotemporal approach with minimal superior transection of dura mater providing scissor-tip access for nerve transection allowed maintaining the integrity of the ophthalmic artery and retinal perfusion.

CONCLUSIONS: This model allows the repair of the optic nerve, preserving the integrity of the ophthalmic artery and retinal perfusion, and therefore is ideal for studies evaluating optic nerve regeneration.

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Delivery of mIR through Biodegradable Scaffolds

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PURPOSE: In severe burns, around 40% patients develop hypertrophic scar contractures (HSc). HSc develop over 6 months and costs millions of dollars annually. HSc are caused by myofibroblasts. MicroRNAs have emerged as key regulators in skin fibrosis, and several candidate miRs (let7c and miR124) have been found to regulate myofibroblast differentiation.

METHODS: We created biodegradable miR delivery scaffolds from poly(CL-co-ethyl ethylene phosphate) (PCLEEP). PCLEEP scaffolds were imaged by scanning electron microscopy. Release kinetics of siRNA were studied. Four groups of scaffolds were implanted in rodents: (1) scaffolds without miR, (2) negative scrambled miRs, (3) scaffolds with 5 μg miR-124, and (4) scaffolds with 5 μg let-7c. Explants from rodents were stained with Masson's trichrome. Fibrous capsules around scaffolds were quantified and graphed.

RESULTS: Scanning electron microscopy analysis demonstrated a fiber diameter ranging from 0.3 to 2 μm. Drug release kinetic studies demonstrated the scaffolds elute miR up to 30 days. Rodent experiments demonstrated that scaffolds incorporate without evidence of extrusion or toxicity. In mice, scaffolds loaded with let7c showed the least capsular formation, followed by miR124 and anti-let7c. In rats, scaffolds loaded with miR124 demonstrated the smallest capsular thickness followed by let7c and anti-let7c.($^*P < 0.05$).

CONCLUSIONS: PCLEEP scaffolds are bioincorporated and useful for drug delivery. Animal experiments with miR loaded PCLEEP scaffolds showed reduction in fibrosis. Ongoing investigations will include analysis of myofibroblast formation, as well as temporal analysis of the inflammatory response.

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The Staged Use of Dermal Regenerate and Spray Skin Technology in the Restoration of Full-Thickness Soft Tissue Defects

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PURPOSE: Full-thickness soft tissue defects secondary to large burns, traumatic, and war-related injuries continue to challenge reconstructive surgeons. Novel approaches are needed to restore the functional, protective barrier, and aesthetic properties of skin. We report the staged use of a dermal regenerate template (DRT; Integra, Integra Lifesciences) with a spray skin epidermal regenerate (ReCell, Avita Medical LLC) in addition to STSG and compare these results with those from patients treated with DRT and STSG alone.

METHODS: First stage is the wound debridement and DRT application to wound beds. In the second stage, 3 to 5 weeks after DRT placement, patients in the control group were treated with STSG, patients in the experimental group were treated with intraoperative application of spray skin and 6:1 meshed STSG. Mechanisms of injury, total defect and treatment sizes, time to complete healing, donor site morbidity/burden, outcomes, and complications were reviewed.

RESULTS: The 2 patients (patient A and patient B) in the control arm had full-thickness wounds of 760 and 2200 cm^2, respectively. Patient A was treated with DRT followed by a 1.5:1 meshed STSG. Patient B was treated with DRT followed by a 3:1 meshed STSG. Both patients healed completely by 7 and 9 weeks, respectively. In the experimental arm (patient C and patient D), wound sizes were 600 and 1190 cm^2, respectively. At the time of spray skin application, patients C and D were treated with 6:1 meshed STSG, both healed completely by 4 weeks after skin grafting.

CONCLUSIONS: We show that staged use of DRT and spray skin provides a safe and effective method for restoring large soft tissue defects, limits donor site morbidity, and decreases donor site burden and time to complete healing. It also allows for a greater mesh ratio and results in matching pigmentation when compared with treatment with DRT and STSG alone. The use of this technology remains promising in patients with significant traumatic, war-related, or large burn injuries who have limited amounts of donor tissue.

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Functional Liver Nodules Develop De Novo in a Cell-Infused SIEA Flap: A Model of Free Flap-Based Organ-Level Tissue Engineering

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PURPOSE: Tissue engineering has been proposed as a solution to the limited donors available for patients with end-stage organ failure. However, strategies aimed at engineering whole organs have been met with significant challenges, arguably the greatest being the difficulties in developing a de novo vascular system. We have previously proposed that microcirculatory beds found within soft tissue free flaps could be used as an autologous source of explantable microcirculation for organ-level tissue engineering. Here, we demonstrate proof of this concept with autoassembly of functional liver nodules within an SIEA flap.

METHODS: SIEA flaps were dissected in Wistar rats. The proximal and distal femoral vessels were clamped to isolate the flap microcirculation. A mixture of 1×10^6 adipose-derived stem cells, 1×10^6 hepatocytes, and 5×10^5 endothelial cells were infused into the flap and allowed to incubate for 2 hours. The flaps were harvested at days 3, 14, and 28 for RNA and protein analysis and histology. Immunohistochemistry was performed looking at liver-specific markers.

RESULTS: Spontaneous autoassembly of liver nodules within the SIEA flaps were noted as early as postoperative day 3. The liver nodules consisted of cells within a sinusoidal architecture that expressed albumin, cytochrome P450 enzymes, α-1-antitrypsin and γ-glutamyl transpeptidase, indicating the presence of cholangiocytes.

CONCLUSIONS: Individual cells perfused into an SIEA flap are capable of autoassembly within the flap into tissue that expresses liver-specific genes and proteins. The use of soft tissue free flaps as an autologous vascularized scaffold is a promising technology for organ-level tissue engineering.

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Articular Cartilage Matrix Formation Using Dynamic Self-Regenerating Cartilage and Photochemical Hydrogels

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PURPOSE: We present a novel method for generating hyaline articular cartilage to improve the outcome of joint surface repair.

METHODS: A suspension of 10×10^6 swine chondrocytes were cultured under reciprocating motion for 14 days. The resulting dynamic self-regenerating cartilage (dSRC) was placed in a cartilage ring and capped with (1) fibrin, (2) collagen, and (3) crosslinked collagen (collagen+hv) gels. A control group consisted of chondrocytes encapsulated in fibrin gel. Constructs were implanted subcutaneously in nude mice and harvested after 6 weeks. Gross, histological, immunohistochemical, and biochemical analysis was performed. In swine femoral condyle, dSRCs were implanted into osteochondral defects capped with collagen gel and compared with defects filled with osteochondral plugs, collagen gel, and left empty after 6 weeks.

RESULTS: In mice, the 3 dSRC groups showed enhanced contiguous cartilage matrix formation over control. Biochemically, the collagen+hv gel dSRC group was statistically improved in glycoaminoglycan content compared with control and 63% of native articular cartilage values. The swine model also showed contiguous cartilage matrix in the dSRC group but not in the collagen gel and empty defects. This demonstrates the survivability and successful matrix formation of dSRC under the mechanical forces experienced by normal hyaline cartilage in the knee joint.

CONCLUSIONS: The results from this study demonstrate that dSRC capped with photochemical hydrogels successfully engineers contiguous articular cartilage matrix in both non-load-bearing and load-bearing environments.

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Use of an Injectable PCL-HA Composite with Tissue Regenerative Matrix to Promote Adipose Tissue Ingrowth in Soft Tissue Defects

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PURPOSE: Reconstructive procedures for soft tissue losses lead to functional and aesthetic disturbances, infection, or fibrosis. Existing injectable materials lack structural integrity to reconstruct large, 3D volumes, fail to regenerate tissue, and offer transient resorption. We present a bioresorbable injectable synthetic that promotes tissue recruitment to promote soft tissue restoration.

METHODS: Polyester nanofibers were cryo-milled to disperse fibers inside hyaluronic acid hydrogels. Solutions were incubated at 37°C for 20 minutes, set for 10 minutes in syringes, and injected into the inguinal fat pad of Lewis rats using a modified serial puncture technique, with a 15-second postinjection massage. Tissues were explanted en bloc at 7, 30, and 90 days, fixed, and stained with Masson's Trichrome and perilipin.

RESULTS: Rapid MSC migration is observed because of optimal porosity and ECM-like ultrastructure. Masson's Trichrome staining demonstrates superior biocompatibility and integration without evidence of inflammation or fibrosis. Injectable composites have enhanced ability to promote MSC differentiation demonstrated by early incorporation at the transformation zone interface. Early histology is indicative of adipogenesis.

CONCLUSIONS: We have developed an injectable, synthetic composite with structural integrity to replace large defects while promoting tissue integration. Our material has long-term permanence, maintains 3D shape and structure, and promotes adipose tissue formation without evidence of foreign body response. Use of our injectable PCL-hyaluronic acid composite has significant implications for permanently reconstituting soft tissue defects.

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Tracking Phenotypic Drift and Osteogenic Capacity of Fresh Adipose-Derived Stromal Cells

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PURPOSE: Studies have employed various cells surface markers to isolate proosteogenic subsets within the larger heterogeneous stromal cell population. This approach, however, has been limited by known phenotypic drift. Thus, we characterized how individual subpopulations change over time and the impact this may have on bone forming capacity.

METHODS: Freshly harvested human adipose-derived stromal cells were analyzed by flow cytometry for markers CD90, CD105, and BMPR-IB. Marker drift was then followed up over 7 days. Positive and negative fractions for each marker were separated and quantum dot labeled before coculturing to define subsequent contributions of each subset to the total population. Finally, osteogenic differentiation of individual subsets from freshly harvested and in vitro cultured cells for 36 hours was evaluated.

RESULTS: Contributions from CD90, CD105, and BMPR-IB positive and negative fractions to the total population dynamically changed over 7 days. $CD90^+$, $CD105^-$, and $BMPR\text{-}IB^+$ cells from freshly harvested lipoaspirate showed enhanced osteogenic differentiation when compared with their counterparts. These differences were more pronounced after 36 hours of culture. Interestingly, a significant number of cells that were $CD90^+$, $CD105^-$, or $BMPR\text{-}IB^+$ at 36 hours were actually derived from $CD90^-$, $CD105^+$, or $BMPR\text{-}IB^-$ cells before plating, with CD-negative cells drifting toward positive and CD-positive cells drifting toward negative.

CONCLUSIONS: Our observations allude to the volatile nature of surface marker expression with in vitro culture. Furthermore, their changing pattern argues against a direct functional role for specific markers in osteogenesis described in previous studies.

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Identification and Characterization of a Long Noncoding RNA Involved in Osteogenic Differentiation

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PURPOSE: Epigenetic regulation plays a key role in specification of cell fate and long noncoding RNAs (lncRNAs) have been shown to regulate chromatin patterns and large-scale transcriptional profiles. Transcriptome analyses have specifically identified multiple candidate lncRNA, which may guide bone differentiation. What role these may play and how they function to promote osteogenesis, however, remains poorly understood.

METHODS: RNA libraries were generated from human adipose-derived stem cells and iPSCs undergoing osteogenic differentiation. Gene ontology (GO) term analysis was performed for differentially expressed transcripts, and guilt-by-association analysis was used to identify lncRNA functionality. Transcriptome integration revealed 1 lncRNA with strong correlation with skeletal development. Osteogenic differentiation after siRNA-mediated suppression of this lncRNA was determined. Subcellular location of the lncRNA was also investigated by nuclear-cytoplasmic fractionation. Finally, chromatin isolation by RNA purification and mass spectrometry was used to determine functional protein-binding partners to elucidate potential mechanism.

RESULTS: Transcriptome integration and GO term analysis identified lncRNA LOC100505806 with unidirectional upregulation during osteogenesis. Confirmation was obtained by quantitative real-time polymerase chain reaction, and knockdown in adipose-derived stem cells significantly reduced bone differentiation, as shown by histological staining and gene expression. Subcellular localization revealed LOC100505806 to be predominantly nuclear. Twenty interacting protein partners were identified using chromatin isolation by RNA purification and mass spectrometry. GO term analysis revealed the proteins to be involved in mRNA processing/stability, RNA localization/splicing, and regulation of gene expression.

CONCLUSIONS: lncRNAs may regulate large-scale transcriptional profiles and represent potential targets to manipulate osteogenic differentiation in multipotent stem cells. These findings also deepen our understanding of regulatory mechanisms guiding acquisition of cell fate.

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Development of a Suture-Less Hernia Mesh with Enhanced Anchoring Strength

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PURPOSE: Ventral hernias are often repaired with surgical mesh anchored by simple interrupted sutures. Recurrence is caused by suture tearing through the tissue, which occurs when the anchor point tensile stress exceeds tissue strength. We propose a polypropylene hernia mesh with seamlessly knit mesh extensions that are sewn into the abdominal fascia to distribute tensile force over a larger surface area, enhance fixation strength, and prevent mesh dehiscence.

METHODS: Tensile testing was performed on a standard of care (SOC) mesh and several prototype meshes with modified variables. All variables were compared with a SOC mesh anchored using simple interrupted 0-Prolene sutures placed at 1-cm intervals. Time to fixation for each group was recorded.

RESULTS: The maximum intraabdominal force that can be generated by a human abdomen is 16 N/cm. In all testing series, our prototype mesh exceeded the 16 N/cm threshold with average UTS of 38.8 ± 2.40 N/cm across all testing parameters. The SOC UTS was below the 16 N/cm threshold with an average of 12.7 ± 1.82 N/cm.

CONCLUSIONS: Our prototype mesh increases anchor point area, decreases tensile stress, and resists failure at physiologic stresses. With anchoring strength greatly above maximum tensile stress, dehiscence is unlikely to occur in vivo. Future studies will include manufacturing a knitted mesh under GMP, benchtop testing of the mesh's mechanical properties, and implantation in an animal model to achieve Food and Drug Administration approval.

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Hyperspectral Imaging for Clinical Assessment of Radiation Dermatitis

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PURPOSE: The relationship between radiation dose and acute radiation dermatitis remains controversial. No objective methods exist to assess degree of skin reaction to radiation. Previously, we have shown that hyperspectral imaging (HSI) can demonstrate that surface hemoglobin readings varied proportionally with radiation dose and skin reactions in an animal model. Correlations between skin reactions, radiation dose, and HSI oxygenated hemoglobin (oxyHb) values were analyzed in this prospective clinical study.

METHODS: Forty-two patients undergoing external beam breast conserving therapy enrolled. Skin radiation doses ranged between 0.2 and 225 cGy per treatment fraction. Baseline hyperspectral images were obtained before starting irradiation in each patient and subsequently before and after each fractionated dose. OxyHb was measured at 3 sites per patient during treatment. Skin reactions were graded at each treatment encounter and compared with cumulative dose and changes in oxyHb.

RESULTS: Each skin reaction score was found to have significantly different average cumulative doses, with skin reaction severity increasing along with increasing dose. Skin reactions had significantly different changes in oxyHb, with increasing changes in oxyHb associated with increasingly severe reactions.

CONCLUSIONS: There is a significant correlation between increasing radiation dose, acquisition of acute dermatitis, and HSI oxyHb. Because clinical stages of acute radiation dermatitis are readily differentiated by oxyHb, HSI could be a reliable assessment tool for an objective skin scoring system during radiation treatment.

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Evaluation of a Fluid-Based Screening Tool for Diagnosis of Heterotopic Ossification Using Cytokine Profiles

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PURPOSE: Heterotopic ossification (HO) occurs in the setting of persistent systemic inflammation. Currently, we lack the ability to predict patients at risk for HO. The goal of this study is to develop a bedside-point-of-care assay to predict those at risk to develop HO, allowing more directed interventions.

METHODS: Serum from C57Bl/6 noninjured and injured mice after Achilles' tenotomy were collected before the injury and 48 hours and 3 weeks after injury. Serum from burn patients before and after HO and a healthy control were also collected. Samples were analyzed for 19 cytokines by Bio-Plex-cytokines-Biorad and reassessed using ELISA assays. The mRNA levels of these cytokines in the injured site were quantified with real-time polymerase chain reaction.

RESULTS: Mouse MCP-1, interleukin-1β, and interleukin-6 levels increased 3.7-, 1.5-, and 1.1-fold change (FC) after the injury. The increase in mice tumor necrosis factor-α was 1.23 FC, and it correlated with the timing of HO formation at 3 weeks postinjury. The level of mice TGF-β increased 1.3 and 1.96 FC at 48 hours and 3 weeks and was associated with an increase of ALP and Runx2 mRNA expression ($P < 0.005$) at the tenotomy site. Human MCP-1 and matrix metalloproteinase 9 levels are higher in the pre-HO stage compared with the control (1 and 1.6 FC) or post-HO stage (123 and 7.9 FC, respectively).

CONCLUSIONS: In this study, we characterized the diagnostic potential of specific cytokines that can serve as biomarkers for an early stage diagnosis of HO. These findings support a possible correlation between serum cytokine levels and HO formation.

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Nonthermal Plasma: Safe, Versatile Technology for Device Sterilization and Wound Healing Applications

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PURPOSE: SteriFreeMed Plasma Processing Technology is a novel, nonthermal plasma (NTP)/free radical, portable device that delivers a highly active reactive oxygen and nitrogen species mixture within a closed-loop system, which rapidly and reliably sterilized cell phones after a single 10-minute NTP treatment, without residual effects on phones. Given the device's remarkable disinfection efficacy, we sought to investigate the potential applicability to living tissue.

METHODS: Two 8-mm, full-thickness, splinted excisional wounds were created on C57bl/6 mice. Mice were treated once for 10 minutes and killed after 7 or 14 days. LIVE/DEAD assays were performed on human umbilical vein endothelial cell, human aortic smooth muscle cell, human fibroblast foreskin, and human placental pericyte in single culture and coculture after 10-minute NTP treatments.

RESULTS: After NTP treatment, there was no gross or histological evidence of residue, aberrant dermal architecture, infection, or edema. By day 7, treated wounds demonstrated statistically smaller wound widths than controls (4.8 ± 0.3 mm vs 7.1 ± 0.4 mm, $P < 0.01$). LIVE/DEAD assays demonstrated 100% cell viability after 10-minute NTP treatments for both single-culture and cocultured combinations of all cells.

CONCLUSIONS: These data indicate that NTP may not only be safely used on living cells and tissue but also it may even augment the wound healing process, indicating the potential for therapeutic application in the biomedical field. SteriFreeMed Plasma Processing Technology has the potential to revolutionize the current practice of device sterilization in the hospital setting and to shift the paradigm of wound healing.

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Short Duration 9-cis-Retinoic Acid Mitigates Postoperative Lymphedema in the Mouse Hindlimb Model

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PURPOSE: Lymphedema is an incurable disease that can occur after lymphadenectomy for the management of malignant tumors. We have previously shown that retinoic acids (RAs) induce lymphangiogenesis in vitro and in vivo and that continuous daily treatment with 9-cis-retinoic acid (9cisRA) for 45 days prevents secondary lymphedema in a mouse hindlimb model. To limit potential toxicity and side effects, we sought to identify the minimum effective duration of 9cisRA treatment.

METHODS: Ten-week-old Balb/c mice were radiated with 20 Gy in the right hindlimb. Ten days later, popliteal lymphadenectomy was performed. Animals were randomized to receive either 0.8 mg/kg 9cisRA or vehicle control through daily intraperitoneal injection for 14 days after surgery. Change in paw thickness relative to the unoperated hindlimb was measured every 5 days for 45 days.

RESULTS: Gross swelling and increased paw thickness were observed in all animals by postoperative day 5. Animals receiving 9cisRA for 14 days had 11.9% less paw thickness relative to the unoperated hindlimb than controls at postoperative day 40 ($P < 0.05$). Paw skin from 9cisRA-treated animals showed significant increase in podoplanin-positive lymphatics relative to untreated controls ($P < 0.05$).

CONCLUSIONS: This study demonstrates that 14 days of 9cisRA is effective in reducing postoperative lymphedema in the mouse hindlimb model. Reduced toxicity and side effects make 9cisRA a viable therapy for prevention of secondary lymphedema.

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Using Text Data Extraction Software to Automate Capture of Adverse Event Data from Electronic Medical Notes

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PURPOSE: Tracking of adverse events (AEs) in clinical practice is usually overseen by quality control officers. This process can be time consuming, labor intensive, and inaccurate as it may depend on remote event recollection by providers and/or chart data interpretation by the officers. In this study, we aimed to (1) prove that a commercially available text data extraction software (TDES) could be used to retrieve patient and AE-specific data from clinical notes entered on our electronic medical record system (EMRS) during standard patient encounters and (2) measure the added provider documentation time (PDT) associated with this "on-the-fly" AE documentation.

METHODS: A 9-line text template was created on our institution's EMRS for documentation of each AE. One hundred AEs were documented within progress notes of 50 fictitious patients. PDT for each AE was measured. To simulate a full month of clinical data, notes without AEs were added, reaching a total of 1000 notes from 100 patients. All notes were then exported (PDF format) and fed into a TDES, which retrieved provider-entered AE data, along with embedded patient demographics and encounter data.

RESULTS: The TDES successfully analyzed the fictitious monthly report containing 1000 encounter notes (3000 pages), generating a database containing 50 patients, 100 AEs, and 2000 data points. AE PDT ranged from 55 to 119 seconds, averaging 82 seconds.

CONCLUSIONS: A commercially available TDES can be used to retrieve patient and AE-specific data from EMRS clinical notes. "On-the-fly" documentation added, on average, less than 1.5 minutes of PDT per AE.

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Redesigned (Data Friendly) Electronic Medical Notes Allow Automated Extraction of Clinical Data and Completion of the American Society of Plastic Surgeons' Annual Statistics Survey

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PURPOSE: The American Society of Plastic Surgeons' Annual Statistics Questionnaire (ASPS-ASQ) is a survey sent to physicians who perform plastic surgery procedures. Those willing to complete the survey face a laborious and time-consuming task. We hypothesized that by using redesigned "data friendly" electronic medical notes (DFNs), text data extraction software (TDES), and spreadsheet software, data could be automatically extracted from providers' operative notes, analyzed, and reconciled for automated reporting of the ASPS-ASQ.

METHODS: One hundred eighteen fictitious operations were created, covering all 259 questions of the 2014 ASPS-ASQ. Each one of them was documented 10 times in our institutions' EMR system as DFNs, which allow customary documentation in prose, but contain specific text prompts mapped to key text data (variables), that can be later recognized/retrieved by a TDES. Sixteen primary variables were assigned for retrieval (patient demographics, procedural specifics). The DFNs were exported to the TDES for data accrual. The resulting database was imported to a preprogrammed Microsoft Excel spreadsheet, to provide specific answers to the ASPS-ASQ.

RESULTS: The TDES analyzed all 1180 operative notes, generating a database containing 29,020 clinical data points (15,550 representing 16 primary variables). This database was analyzed by our Excel spreadsheet, which was able to provide specific answers to all questions in the 2014 ASPS-ASQ, except for those about cosmetic fees (55) and recurrent aesthetic patients.

CONCLUSION: By using DFN design for operative notes, TDES, and spreadsheet software, we were able to automate reporting for the 2014 ASPS-ASQ, with the exception of questions on physician fees and recurrent patients.

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Validation of a Novel Model of Recurrent Heterotopic Ossification

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PURPOSE: Current treatment strategies for heterotopic ossification (HO) include surgical extirpation. However, excision rarely alleviates the long-term sequelae of HO such as chronic pain and open wounds, and these patients often suffer from recurrence. Here, we describe a new model of recurrent HO, which reexpresses the signaling mediators present during initial HO formation.

METHODS: Wild-type C57BL/6 mice underwent 30% TBSA burn with Achilles' tenotomy to induce HO at the tenotomy site. After 9 weeks, mice underwent baseline micro computed tomography followed by HO excision and immediate reimaging within 72 hours. Mice were allowed to survive an additional 9 weeks, at which time repeat imaging was performed. Immunostaining for pSMAD1/5, a known mediator of bone morphogenetic protein signaling, was performed in a subset euthanized 3 weeks after excision.

RESULTS: By micro computed tomography, we identified the presence of new ossified lesions occurring postexcision (A). Alcian blue and immunostaining of samples 3 weeks after initial burn/tenotomy showed substantial cartilage and pSMAD 1/5 expression. However, 9 weeks after initial burn/tenotomy, cartilage and pSMAD1/5 were almost eliminated. In post-excisional HO, recurrence of new cartilage and pSMAD1/5 expression was noted by 3 weeks (B).

CONCLUSIONS: We have designed a model of recurrent HO after surgical excision. Similar signaling mediators as during initial HO were upregulated, including pSMAD 1/5. Patients who undergo HO excision may benefit from therapeutics that are currently being evaluated to prevent initial HO.

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Differential Gene Expression in Capsules from Textured Versus Smooth Silicone Implants

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PURPOSE: Capsular contracture complicates implant-based breast surgery leading to abnormal appearance, increased firmness, and pain to touch. It is known that textured implants reduce the rate of capsular contracture. This study examines RNA expression in a rat model of capsular formation around textured and smooth silicone implants to understand contracture pathogenesis and identify therapeutic targets.

METHODS: Miniature round smooth or textured silicone devices were implanted into a subcutaneous flank pocket of Fisher rats (n = 10). After 6 weeks, the capsule was explanted and examined by histological and molecular techniques. Differential gene expression was identified using RNA sequencing. Selected gene expression levels were confirmed by quantitative real-time polymerase chain reaction and immunohistochemical staining.

RESULTS: RNA sequencing data were subjected to the Probability of Positive Log Ratio (PPLR) algorithm. Transcripts were identified for further characterization using cutoff values of PPLR $\geq$ 0.975 (2-fold increase) or a PPLR $\leq$ 0.025 (2-fold decrease). We identified 18,555 transcripts, and 404 transcripts met PPLR inclusion criteria. Quantitative real-time polymerase chain reaction was performed for matrix metalloproteinase 3, troponin 3 (TNNT3), and neuregulin 1. metalloproteinase 3 and TNNT3 are downregulated in smooth implant capsules with a fold difference of −2.04 ($P < 0.006$) and −3.03 ($P < 0.0056$), respectively. Neuregulin 1 is upregulated in smooth implant capsules with a fold difference of +2.65 ($P < 0.0001$). Immunohistochemical staining was consistent with differential expression patterns.

CONCLUSIONS: We demonstrate that capsules around smooth and textured implants have different histological appearances and different patterns of gene expression. These differences may explain the decreased rate of contractures seen surrounding textured implants.

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Photochemical Tissue Passivation Attenuates Intimal Hyperplasia in an Arteriovenous Fistula Model

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PURPOSE: Although arteriovenous fistulae (AVF) are the gold standard for vascular access, their effectiveness is limited by poor patency; 40% to 70% of fistulae fail within 1 year because of intimal hyperplasia (IH). Venous stretch injury from exposure to arterial pressure induces IH. Photochemical tissue passivation (PTP) is a novel technology that cross-links adventitial collagen, strengthening the vein and decreasing its compliance to resemble that of an artery. We hypothesize that decreasing vein compliance will protect the vein against stretch injury and reduce IH.

METHODS: AVF were created between the femoral artery and superficial epigastric vein in Sprague-Dawley rats. PTP was performed immediately before performing vessel anastomosis. AVF were harvested after 4 weeks, and each specimen was divided into 3 regions: the venous juxta-anastomotic segment, proximal vein, and distal vein. Venous diameter was measured at the time of the initial procedure and at harvest. Intimal area was measured for each segment.

RESULTS: All AVF remained patent. Dilatation of the venous segments of AVF at initial placement/1 month were 27% ± 7%/45% ± 28% for PTP-treated vessels and 76% ± 17%/112% ± 33% for controls ($P = 0.02$). Intimal area was $0.160 \pm 0.084\,mm^2$ and $0.028 \pm 0.023\,mm^2$ for control and PTP-treated juxta-anastomotic segments, respectively ($P = 0.02$). Total intimal area for control and PTP-treated AVF was $0.266 \pm 0.099\,mm^2$ and $0.094 \pm 0.051\,mm^2$, respectively ($P = 0.04$).

CONCLUSIONS: PTP treatment effectively reduces juxta-anastomotic and total IH by improving vein elasticity to attenuate venous stretch injury.

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Disparities between Operative Time and Relative Value Units for Plastic Surgery Procedures

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PURPOSE: Plastic surgeons are evaluated not only by the number of patients served but also by relative value, quantified by the Medicare Relative Value Unit (RVU) system, which can affect advancement and compensation. Procedures that demand a longer operative time without an increase in RVUs are, by definition, inefficient. The purpose of this study is to determine whether the number of RVUs actually corresponds to operative time.

METHODS: NSQIP datasets from 2005 to 2013 were queried for plastic surgery operations, and the time to RVU ratio (TRR) was calculated for each operation. The primary Current Procedural Terminology codes representing the 100 most common surgeries were compared for operative time, total RVUs, and TRR.

RESULTS: Inclusion criteria yielded 53,696 patients. There was a high degree of correlation between operative time and number of RVUs ($r^2 = 0.82$). Excisions of sacral pressure ulcers had the lowest TRR, indicating the highest level of surgical efficiency. Infected skin debridement had the highest TRR, indicating the lowest efficiency. The average TRR was 8.54 minutes per RVU.

CONCLUSIONS: As a general trend, the most common plastic surgical procedures requiring longer operative times are associated with more RVUs. The cases with lower TRRs tended to have higher operative times and RVUs, implying that surgeons selecting more involved cases were rewarded with an increase in relative value.

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Impact of Terminal Schwann Cells on Functional Recovery after Nerve Injury

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PURPOSE: Terminal Schwann cells (tSCs) are a special type of supportive glial cell that reside at the neuromuscular junction and are particularly active during reinnervation. After motor nerve injury, imaging studies have demonstrated that tSCs induce nerve sprouting and extend elaborate processes through which regenerating axons are able to grow. The implications of tSCs on functional recovery, however, are unknown. By ablating tSCs, we sought to determine the importance of tSCs on functional recovery after motor nerve injury.

METHODS: Thirty-two Thy1-CFP/S100-GFP mice were randomized into 4 groups (n = 8 per group: 5 experimental, 3 controls) corresponding to time points at which functional testing was performed after sciatic nerve transection and repair. Twenty-four hours after nerve injury, tSCs were ablated with GD3 antidisialosyl antibodies into the extensor digitorum longus muscle. We then evaluated the morphology of the neuromuscular junction (NMJ) and muscle force generated by the extensor digitorum longus at t = 1, 2, 4, and 6 weeks after injury.

RESULTS: Preliminary results demonstrate reduced muscle force after ablation of tSCs at all 4 time points after nerve injury. Confocal microscopy images demonstrate the absence of tSC processes and reduced nerve sprouting.

CONCLUSIONS: Ablation of tSCs after nerve injury results in significantly reduced muscle force and morphologic changes of the NMJ. These data suggest that tSCs are obligate participants in NMJ reinnervation after motor nerve injury.

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Regenerative Peripheral Nerve Interface Muscle Graft Size Influences Optimal Signal Transduction

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PURPOSE: Regenerative peripheral nerve interfaces (RPNIs) successfully form bioartificial interfaces between severed nerves and electrical neuroprostheses. Accepted surgeries use muscle grafts of approximately 130 mg; however, the optimal grafted volume for electrical signaling has not yet been determined. This study evaluates RPNI electrical signaling capacity with respect to increasing grafted skeletal muscle volume.

METHODS: F344 rats (N = 24) were assigned to 1 of 4 groups defined on implanted muscle volume: 150 mg (RPNI 150), 300 mg (RPNI 300), 600 mg (RPNI 600), or 1200 mg (RPNI 1200). Each RPNI consisted of a semimembranosus allograft neurotized by the transected peroneal nerve. RPNIs were analyzed 3 months postsurgery for compound muscle action potential, force, and histology.

RESULTS: Retained muscle tissue volume was greater in RPNI 150 (41.7% ± 3.7%) and RPNI 300 (29.8% ± 1.1%) compared with RPNI 600 (21.9% ± 1.7%) and RPNI 1200 (15.0% ± 1.1%; $P < 0.05$). RPNI 150 showed a greater compound muscle action potential compared with RPNI 1200 (6.6 ± 1.3 mV vs 2.3 mV ± 0.7 mV, $P < 0.05$). Percentage of viable tissue was highest for RPNI 150 and declined with larger RPNIs ($P < 0.001$).

CONCLUSIONS: Small RPNIs of 150 to 300 mg are optimal for RPNI electrical signaling in a rat model. This is favorable because small muscle grafts will allow for implantation of multiple RPNIs to increase the number of outputs for voluntary control.

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Dermal Sensory Interfaces for Providing Feedback to Amputees

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PURPOSE: We developed a dermal sensory interface (DSI) with the ultimate goal of providing amputees with meaningful sensory feedback through the residual nerve. Our purpose was to (a) determine predictability of afferent nerve action potentials evoked by electrical stimuli delivered to DSIs; and (b) verify that sensory peripheral nerves neurotize DSIs implanted subcutaneously without forming neuromas.

METHODS: Rat hindlimbs were assigned to 1 group: (a) control full-thickness skin (n = 10), (b) control deepithelialized skin (n = 10), (c) control sural nerve (n = 10), and (d) DSI (n = 10). Each DSI was constructed by surrounding the residual sural (sensory) nerve with an autologous deepithelialized glabrous skin graft. After 2 months' convalescence, patterned electrical stimuli were applied, and evoked responses were recorded at the sural nerve.

RESULTS: All DSIs showed healthy revascularization. Over 96% of pulses delivered to DSIs at 100 A above absolute current threshold elicited graded compound sensory nerve action potentials at frequencies ≤100 Hz. Three-dimensional microscopy visualized robust reinnervation of DSIs originating from transected sural nerve fibers without formation of neuroma.

CONCLUSIONS: Electrical stimuli of varying frequency and amplitude reliably evoke graded sensory nerve feedback from DSIs, and sensory fibers regenerate throughout DSI constructs without signs of neuroma.

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Electrical Muscle Stimulation Does Not Influence Synkinetic Reinnervation in a Novel Rat Model of Peripheral Nerve Injury and Repair

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PURPOSE: Recovery after nerve repair is limited by axon misdirection. Studies have shown that electrical muscle stimulation (EMS) after nerve repair reduces synkinetic reinnervation. Conclusions were based on functional measurements with no direct evidence. Here, we used a novel rat model to directly investigate whether EMS increases the proportion of motoneurons reinnervating original targets after injury.

METHODS: The original soleus motoneuron pool was labeled with True-Blue. After 1 week, the right lateral gastrocnemius soleus nerve was transected and repaired, and the soleus muscle was implanted with electrodes. One group received EMS with a natural activation paradigm. Two months later, the right soleus nerve was retrograde labeled with Fluoro-Ruby to label regenerated motoneurons. The contralateral uninjured lateral gastrocnemius and soleus nerves were labeled with True-Blue and Fluoro-Ruby, respectively, to compare the spatial distribution of the contralateral motoneuron pool with the injured side. The number of regenerated axons was counted with histomorphometry.

RESULTS: Three-dimensional reconstructions of labeled motoneuron pools demonstrated the majority of motoneurons regenerating to the soleus muscle derived from the lateral gastrocnemius motoneuron pool. EMS did not increase the proportion of original soleus motoneurons regenerating toward the soleus muscle (EMS: 10.9% ± 2.3% vs Sham: 15.5% ± 9.9%). There were no differences in axons counts (EMS: 200 ± 68 vs Sham: 163 ± 47).

CONCLUSIONS: Although studies suggest that EMS reduces muscle atrophy, our model does not support the use of EMS to reduce axonal misdirection after nerve repair.

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Femoral Nerve Transfers for Restoring Tibial Nerve Function

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PURPOSE: Sciatic nerve injuries cause debilitating functional impairment, particularly when injury mechanism precludes reconstruction using primary grafting. The purpose of this study is to demonstrate anatomic feasibility of nerve transfers from distal femoral nerve branches to tibial nerve and describe successful restoration of tibial nerve function in clinical practice using the described lower extremity nerve transfer.

METHODS: Six cadaver legs were dissected for anatomic analysis and development of tension-free nerve transfers from femoral nerve branches to the tibial nerve. In 2 patients with complete sciatic nerve palsy, terminal branches of the femoral nerve supplying vastus medialis and vastus lateralis were transferred to medial and lateral gastrocnemius branches of the tibial nerve. Distal sensory transfer of the saphenous nerve to the sural nerve was also performed. Patients were clinically assessed for lower extremity motor and sensory recovery up to 18 months postoperative.

RESULTS: Consistent branching patterns and anatomic landmarks were present in all dissection specimens, allowing for reliable identification, neurolysis, and capitation of donor femoral and saphenous nerve branches to the recipients. In the clinical population, patients obtained Medical Research Council grade 3 and 3+ plantar flexion by 18 months postoperatively. Improved strength was accompanied by improved ambulation in both patients and a return to competitive sport in 1 patient. Sensory recovery was demonstrated in both patients by an advancing Tinel sign.

CONCLUSIONS: This study illustrates clinical success and anatomic reliability of femoral nerve to tibial nerve transfers after complete sciatic nerve injury.

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Prospective Evaluation of Outcomes in Migraine Surgery Patients at the Massachusetts General Hospital

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PURPOSE: Several groups have shown positive responses to migraine surgery in 68% to 95% of cases. However, most studies have analyzed data retrospectively. Few groups have presented prospective outcomes. We present the first prospective data at the Massachusetts General Hospital.

METHODS: Forty patients were prospectively enrolled and asked to complete information on demographics, medical history, and migraine history and to answer the following questionnaires: Migraine headache index [MHI; migraine headache frequency (days) × duration (fraction of 24hours) × average pain severity (on a scale of 0–10)], Headache impact test (HIT6), and pain self-efficacy questionnaire. Results were analyzed after a 12-month follow-up.

RESULTS: Mean MHI, HIT6, and pain self-efficacy questionnaire scores changed significantly from preop to postop. Interestingly, there was no difference between 3 and 12 month results. The mean MHI improvement was 78% at 3 months and 88% at 12 months. At 12 months, the MHI in 30 (80%) of patients improved >80%, 6 (15%) improved between 50% and 80%, and 2 (5%) improved <50%. There were no major adverse events.

CONCLUSIONS: Results in our prospective series are comparable with our retrospective results, further supporting the safety and efficacy of migraine surgery as a treatment option for migraine sufferers. No significant difference in outcomes between 3 and 12 months suggests that results can be predicted at 12 weeks.

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A Glial Cell Line–Derived Neurotrophic Factor Delivery System Enhances Nerve Regeneration across Acellular Nerve Allografts

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PURPOSE: Acellular nerve allografts (ANAs) are used clinically to bridge nerve gaps. We questioned whether the ability of these allografts to support nerve regeneration could be improved by supplementation with key neurotrophic factors. Here, we investigated a local drug delivery system (DDS) for glial cell line-derived neurotrophic factor (GDNF)-controlled release to implanted ANAs in rats using drug-loaded polymeric microspheres (MS) embedded in a fibrin gel.

METHODS: In a rat hindlimb nerve gap model, a 10-mm ANA was used to bridge a 5-mm common peroneal nerve gap. Experimental groups received DDS treatment at both suture sites of the allografts releasing GDNF for either 2 or 4 weeks. In negative control groups, rats received no DDS treatment or empty DDS. Rats receiving nerve isografts served as the positive control group. Eight weeks after repair, nerve regeneration was assessed using retrograde labeling and collecting nerve samples 10 mm distal to the graft for histomorphometric analysis.

RESULTS: The numbers of motor and sensory neurons that regenerated their axons in all the groups with GDNF MS and isograft treatment were indistinguishable. These numbers were significantly higher compared with the negative control groups. Nerve histology distal to the nerve graft demonstrated increased axon counts. Fiber frequency analysis indicated a shift to larger fiber diameters because of GDNF MS treatment.

CONCLUSIONS: The sustained delivery of GDNF to the implanted ANAs achieved in this study demonstrates the promise of this DDS for the management of severe nerve injuries with large nerve defects.

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In Search of a Terminal Schwann Cell-Specific Marker: Does a Tool Exist?

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PURPOSE: Specialized structures at the neuromuscular junction (NMJ) facilitate the interface between nerve and muscle. Terminal Schwann cells (tSCs) are glial cells present at the NMJ. Although these nonmyelinating Schwann cells have been implicated in multiple functional roles, there is no known marker specific to tSCs, making isolation and investigation of this cell type challenging. We sought to identify genetic markers unique to tSCs.

METHODS: A novel component dissection technique was utilized to isolate the tSC-containing endplate band from the sternomastoid muscles of young adult *S100-GFP* mice. RNA was isolated from samples containing (1) endplate bands (tSCs + nerve + muscle), (2) nerve, and (3) muscle and prepared for microarray analysis. Rank-order analysis was performed to identify genes specific to tSCs.

RESULTS: Preliminary data have generated a total of 11 genes unique to the NMJ. Our short list of candidate genes specific to tSCs includes *D-2-hydroxyglutarate dehydrogenase* (*D2hgdh*), *Collagen Q* (*ColQ*), and *T-bet 21* (*Tbx21*). These genes were upregulated at the NMJ by 4- to 11-fold compared with muscle or nerve parts alone ($P < 0.05$). D2hgdh protein expression colocalizes with tSCs and is not noted in myelinating SCs from sciatic nerve. Current validation studies of these candidate genes are ongoing.

CONCLUSIONS: Given their unique functional roles, tSCs likely have a unique transcriptome that differentiate them from other Schwann cell types. Identification of genetic specificity for tSCs facilitates improved methods to investigate these unique cells and ultimately allows for transgenic mouse line creation that permits Cre-mediated recombination in tSCs.

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Functional Outcomes and Histomorphometric Analysis of Donor Axon Regeneration after Facial Reanimation with Cross-Face Nerve Grafts and Gracilis Muscle Transplants in Pediatric and Adult Facial Palsy Patients

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PURPOSE: Facial reanimation with cross-face nerve grafts is the gold-standard treatment in severe and long-standing cases of facial palsy. We hypothesized that the functional and histomorphometric results after cross-face nerve grafting and gracilis muscle transplantation are superior in the pediatric patient group when compared with adults.

METHODS: Nineteen pediatric patients (<18 years of age) and 48 adult patients undergoing facial reanimation surgery with cross-face nerve grafts (first stage surgery) and gracilis muscle transplantation (second stage) with postoperative follow-up times of ≥18 months were included in the study. Standardized facial movements were recorded for 3D video analysis preoperatively and postoperatively in all patients. Nerve biopsies were available in 9 pediatric patients (53%) and 13 adults (27%). Descriptive statistical analysis, *t* tests and nonparametric tests were performed, and correlations (Spearman-Rho coefficient) were calculated; α was set at 5%.

RESULTS: 3D video analysis showed significant functional improvement 18 months postoperatively in both the pediatric and the adult groups. The symmetry of "smiling with showing of the teeth" was significantly higher in pediatric patients. The improvement of eye function failed to show statistically significant differences between groups. The axon count of the facial nerve donor branches and the percentage of axons that reached the distal end of the cross-face nerve graft did not correlate with the patient age and did not significantly differ between the 2 groups.

CONCLUSIONS: Facial function was significantly improved in all patients. The reanimation of the mouth was more symmetrical in pediatric patients, although the axon count did not differ.

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Velopharyngeal Dysfunction and Sleep Apnea: A Survey to Ascertain Surgical Practice Patterns

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PURPOSE: Velopharyngeal dysfunction (VPD) results in poor contact between the palate and pharynx, leading to abnormal speech. In addition, patients with VPD often have obstructive sleep apnea (OSA). This particular study sought to determine what variables impact a surgeon's investigation into OSA during VPD treatment.

METHODS: A 22-question survey was administered via e-mail to 1117 surgeons who were members of the American Cleft Palate-Craniofacial Association. Logistic regression was used to determine whether management was affected by years in practice, clinical volume, field of training, and region of practice.

RESULTS: Two hundred thirty-one surgeons responded (21% response rate), and 67% stated they trained in plastic surgery. With increasing years of practice, surgeons were less likely to refer patients for preoperative and postoperative sleep studies ($P = 0.00$ and $P = 0.001$, respectively), screen patients for sleep apnea ($P = 0.008$), or change their management based on a sleep study ($P = 0.001$). There were no significant differences in screening or testing for OSA based on clinical volume. Among those surveyed, otolaryngologists were more likely to refer patients for postoperative sleep studies ($P = 0.028$). Surgeons in the Southeast were more likely to change their management based on a sleep study ($P = 0.038$).

CONCLUSIONS: Statistically significant trends in screening and testing for OSA in the setting of VPD were identified by this survey. On the basis of the results of this study and the increasing literature linking OSA and VPD, we suggest an organized effort to help educate practicing cleft surgeons on the potential ramifications of VPD surgery on a child's airway.

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Use of Biologic Tissue Matrix in Postneurosurgical Posterior Trunk Reconstruction Is Associated with Higher Wound Complication Rates

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PURPOSE: Patients undergoing neurosurgical spine surgery for spinal tumors are increasingly undergoing soft tissue reconstruction involving biologic tissue matrices. There are limited data available on the safety of these devices in posterior trunk reconstruction.

METHODS: A cohort study of patients undergoing oncologic spine surgery with subsequent plastic surgery reconstruction was conducted. The primary outcome variable was development of a postoperative wound complication, whereas secondary outcome variables were specific complications.

RESULTS: Two hundred ninety-three cases in 260 patients were included. The matrix and nonmatrix cohorts were similar in regards to demographic, medical, and surgical variables. The rate of all-cause wound complications in patients receiving biologic matrix for reconstruction was 49.2%, whereas the all-cause complication rate for patients not receiving the matrix was 31.7% ($P = 0.010$). The rates of infection (34.9% vs 20.9%) and seroma (19.0% vs 10.0%) were also increased in patients receiving biologic matrix. In multivariate analysis, biologic matrix use remained a predictor of wound complications ($P = 0.045$), infection ($P = 0.011$), and seroma ($P = 0.047$).

CONCLUSIONS: We identified an increased risk of infection and seroma with the use of biologic tissue matrix in posterior trunk reconstruction even after controlling for reconstruction type and demographics. Careful consideration of the risks and benefits of using these devices in this population is warranted.

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Postoperative Antibiotic Prophylaxis in Reduction Mammaplasty: Preliminary Results of a Randomized Controlled Trial

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PURPOSE: Despite reduction mammaplasty is classified as a clean operation, previous studies have demonstrated that the postoperative use of antibiotics is beneficial for patients. However, there is no scientific evidence to support the use of antibiotics after reduction mammaplasty. This 2-arm parallel-group randomized controlled trial was designed to assess the influence of postoperative antibiotic prophylaxis on surgical site infection (SSI) rates after reduction mammaplasty.

METHODS: The calculated sample size was 62 patients per arm. We present preliminary results of 80 patients. All patients received cephalotin, 1 g at the anesthetic induction and at each 6 hours, for the first 24 hours. Breast hypertrophy patients undergoing reduction mammaplasty were prospectively enrolled. All patients received cephalotin, 1 g at the anesthetic induction and at each 6 hours, for the first 24 hours. At the hospital discharge, patients were randomly allocated to placebo group (n = 40), which received a capsule of placebo for 7 days, each 6 hours, or to antibiotic group (n = 40), which received cephalexin, 500 mg each 6 hours, for 7 days. SSIs were defined by standard criteria from the Centers for Disease Control and Prevention. Patients were assessed weekly by a blinded surgeon, for 1 month.

RESULTS: At baseline, groups were similar with regard to age ($\rho = 0.211$), body mass index ($\rho = 0.218$), weight of resected breast tissue ($\rho = 0.314$), and duration of operation ($\rho = 0.364$). None of the patient, in both groups, developed SSI.

CONCLUSIONS: These preliminary results demonstrated no differences in SSI rates between groups, suggesting that the postoperative use of antibiotics could be unnecessary.

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Thirty Days of Reporting in Periprosthetic Breast Infections Is Not Enough

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PURPOSE: Current national databases, such as the National Surgical Quality Improvement Program, only report 30-day postoperative outcomes. Thus, many breast implant-related complications go unreported in standard databases. We sought to characterize late periprosthetic infections after implant-based breast reconstruction.

METHODS: We conducted a retrospective analysis of all women undergoing expander/implant breast reconstruction from 2005 to 2014 at 2 institutions. Periprosthetic infections were defined as any episode when antibiotics were initiated or when a prosthetic device was explanted because of infection, and they were classified as occurring early (≤30 days) or late (>30 days).

RESULTS: Of the 1820 patients and 2980 breasts identified, 421 periprosthetic infections occurred (14%). Of these, 173 (41.1%) were early and 248 (58.9%) were late (mean time to infection = 66.4 ± 101.9 days). Patients with late infections were more likely to be current smokers or diabetics than patients with early infections ($P < 0.034$ for both). Infections caused by Gram-negative bacteria and antimicrobial-resistant strains of *Staphylococcus* were more common in the early infection group ($P < 0.001$ for both). Implant loss because of infection was more common in the late infection group ($P = 0.037$).

CONCLUSIONS: Late periprosthetic infections after implant-based breast reconstruction are underestimated in national outcome databases and have unique risk factors and microbiology compared with early infections. A system-level change in reevaluating and redefining a timeline for tracking and treating implant infections is necessary given the substantial morbidity associated with, and frequency of, late periprosthetic infections.

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Hand Function and Appearance after Reconstruction for Congenital Hand Differences: A Qualitative Analysis

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PURPOSE: Congenital hand differences induce social, psychological, and functional challenges for children. However, little is known about how children perceive their outcomes after reconstructive procedures or what concerns children have.

METHODS: A total of 33 children (age, 6–17 years), who were treated for congenital hand abnormalities, and their parents participated in qualitative, semistructured interviews regarding the child's hand function and appearance. Discussion focused on the influence of congenital hand differences on the child's daily activities, school, and participation in sports and music. The interviews were open format to allow for the spontaneous emergence of relevant themes followed by guided questioning. The interviews were transcribed verbatim and analyzed using qualitative coding, iterative comparisons, and frequency analysis to reveal perceptions of children and parents.

RESULTS: In this sample, 73% of children and parents reported difficulty with hand function. Children experienced difficulties with personal care (58%), school activities (30%), and household tasks (27%). Children were also bothered by their hand appearance (48%), pain (30%), and weakness (24%). Complex anomalies were associated with greater disability and limitation in sports and music.

CONCLUSIONS: Children with congenital hand differences are concerned with the aesthetic appearance of their hands and limitations in their ability to perform activities. Children were often discouraged by activities that their peers accomplished easily, but with increasing age demonstrated adaptive behaviors to accommodate in their "own way," suggesting the uniqueness of their limitations. Patients may benefit from early hand therapy evaluation guided toward areas of concern to enhance functional adaptation for activities and tasks.

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The Effect of Antibiotic and Drain Duration on Infectious Complications after Alloplastic Breast Reconstruction

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PURPOSE: Currently, there is no evidence-based standard for postoperative antibiotic or drain duration after alloplastic breast reconstructions. In this study, we investigate infectious outcomes after alloplastic breast reconstruction in 2 different antibiotic duration groups.

METHODS: This retrospective cohort study investigated all patients undergoing alloplastic breast reconstruction at a single institution from 2010 to 2012. During this period, some patients were enrolled in a trial where antibiotics were discontinued after 24 hours. All other patients had antibiotics continued until drain removal. Both study and nonstudy patients from the time period were included and compared by antibiotic and drain duration, comorbidities, demographics, and infectious complications. Statistical analysis was performed via generalized linear mixed model.

RESULTS: Our cohort included 282 patients with 467 reconstructions. There was no significant difference in demographics and comorbidities among antibiotic groups. The rates of cellulitis and deep space infection in both study groups was 10.7%. No significant difference was found in cellulitis rates between the antibiotic groups ($P = 0.713$), although patients in the 24-hour antibiotic use were found to have lower rates of deep space infection (odds ratio = 0.329, $P = 0.0386$). Prolonged drain duration (>2 weeks) was associated with both cellulitis and deep space infection rate ($P = 0.0015$ and 0.0007, respectively) while having no significant effect on seroma occurrence ($P = 0.1269$).

CONCLUSIONS: These results show that drain duration longer than 2 weeks may contribute to postoperative infections while having no effect on seroma occurrence. Prolonging the course of antibiotics does not appear to reduce infectious complications, although further randomized prospective trials are needed.

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Incidence and Risk Factors of Major Complications in Brachioplasty: Analysis of 2294 Patients

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PURPOSE: Brachioplasty is a popular procedure to correct upper arm ptosis. However, current literature on complications and risk factors is scant and inconclusive. By using a large, prospective, multicenter database, we report the incidence of major complications and risk factors in patients undergoing brachioplasty.

METHODS: Patients who underwent brachioplasty between 2008 and 2013 were identified from the CosmetAssure database. The primary outcome was the occurrence of major complication(s), defined as complications requiring emergency room visit, hospital admission, or reoperation within 30 days of the procedure. Risk factors including age, gender, body mass index (BMI), smoking, diabetes, combined procedures, and type of surgical facility were evaluated using univariate and multivariate analysis.

RESULTS: Within the 129,007 patients enrolled in CosmetAssure, 2294 (1.8%) underwent brachioplasty. Brachioplasty patients were more likely to be older than 50 years (50.1%), obese (36.3%), and diabetic (5.5%) but less likely smokers (5.5%). Major complications occurred in 3.4% brachioplasties with infection (1.7%) and hematoma (1.1%) being most common. Combined procedures, performed in 66.8% cases, had a complication rate of 4.4%, in comparison with 1.3% for brachioplasties performed alone. Combined procedures (RR = 3.58), male gender (RR = 3.44), and BMI $\geq 30\,kg/m^2$ (RR = 1.92) were identified as independent risk factors for the occurrence of any complication. Combined procedures (RR = 12.42) and the male gender (RR= 8.89) increased the risk of hematoma formation.

CONCLUSIONS: Complication rates from brachioplasty are much lower than previously reported. Hematoma and infection are the most common major complications. Combined procedures, male gender, and BMI $\geq 30\,kg/m^2$ are independent risk factors for complications.

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Thighplasty: Complication Rates, Risk Factors, and Analysis of Concurrent Cosmetic Surgical Procedures

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PURPOSE: Despite an increase in thighplasty, outcomes and risk factors have not been well described. This study investigates the incidence and risk factors of major complications and the safety of combining procedures with thighplasty in a large, prospective, multicenter database.

METHODS: Patients undergoing thighplasty between 2008 and 2013 were identified within the CosmetAssure database. The primary outcome was the occurrence of major complication(s) requiring emergency department visit, hospital admission, or reoperation within 30 days postoperatively. Age, gender, body mass index, smoking, diabetes, type of facility, and combination procedures were evaluated as risk factors.

RESULTS: Among the 129,007 patients enrolled in CosmetAssure, 1493 (0.8%) underwent thighplasty. One thousand eighty-eight (72.9%) thighplasties were combined with other procedures. Ninety-nine (6.6%) developed at least 1 complication. The most common complications were infection (2.7%), hematoma (2.1%), suspected/confirmed VTE (1.4%), and fluid overload (0.5%), which were higher than nonthighplasty patients. Hospital-based thighplasties had higher complications (8.1%) than ambulatory surgical center (6.2%) and office-based surgical suite (3.1%). When thighplasty was performed alone, smoking was an independent risk factor to develop at least 1 complication (RR = 9.51) and hematoma (RR = 13.48). Concomitant lower body lift increased complication risk (11.8% vs 4.7%).

CONCLUSIONS: Thighplasty has a higher major complication rate compared with most other cosmetic procedures, but lower than previously thought. In thighplasty alone, smoking is the only independent risk factor for overall complications and hematoma formation. Thighplasties performed in office-based surgical suite have lower complications rates. There was no complication difference between single or combined procedures except when lower body lift was included as a concomitant procedure.

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Analyzing Treatment Aggressiveness and Identifying High-Risk Patients in Diabetic Foot Ulcer Return to Care

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PURPOSE: Plastic and reconstructive surgery techniques are often required in managing the complex wounds that result from diabetic foot ulcers. This retrospective study analyzed subjects with diabetes mellitus and distal foot ulcers to determine the factors that predict hospital readmission.

METHODS: The cohort was constructed from patient discharges containing diagnosis codes for both diabetes mellitus and distal foot ulcer. Data were collected from the State Inpatient Database and State Emergency Department Database from the Agency for Healthcare Research and Quality in Florida and New York, 2011 to 2012. All-cause 30-day return to care admissions (emergency room or inpatient admission) were identified.

RESULTS: Our cohort included 25,911 patients, of whom 21% underwent a toe or midfoot amputation during their index stay. The whole cohort rate of return to care within 30 days was 30%, whereas the toe or midfoot amputation group had a significantly lower readmission rate of 25% ($P < 0.05$). The most common diagnosis codes on readmission were diabetes mellitus (19%) and infection (13%). In a multiple regression model, patients with a toe or midfoot amputation procedure had 22% lower odds of readmission ($P < 0.05$). Other demographic factors such as comorbidities, black and Hispanic ethnicities, and Medicare and Medicaid payers were associated with higher odds of readmission after initial hospitalization ($P < 0.05$).

CONCLUSIONS: The results of this study suggest that aggressive management of diabetic foot ulcer patients may decrease odds of return to inpatient or emergency department care. Understanding patients at high risk for readmission can improve counseling and treatment strategies for this fragile patient population.

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Health-Related Quality of Life in Patients Undergoing Body Contouring Plastic Surgery after Massive Weight Loss—A Systematic Review and Meta-Analysis

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PURPOSE: Evidence exists regarding functional and aesthetic benefits of body contouring plastic surgery (BCPS) after massive weight loss. Study objective was to perform a systematic review and meta-analysis to assess health-related quality of life (HRQoL) after BCPS in massive weight loss population.

METHODS: A literature search was performed using the databases OVID-Medline, Institute of Scientific Information Web of Science, and EMBASE. The meta-analysis was conducted using the random effects model. Publication bias was explored using funnel plot and the Egger regression intercept.

RESULTS: A total of 814 studies were identified. Thirty studies addressed HRQoL and BCPS, of which 10 were included in the meta-analysis. Pooled improvement in overall HRQoL was 0.63 [95% confidence interval (CI), 0.42–0.84; $P < 0.0001$]. HRQoL domains who presented the highest improvement were body image (1.02; 95% CI, 0.75–1.3; $P < 0.001$) and social (0.67; 95% CI, 0.4–0.94; $P < 0.001$). Modest improvement was found in pain and work HRQoL domains (0.37; 95% CI, 0.02–0.76; $P = 0.06$ and 0.38; 95% CI, 0.08–0.68; $P = 0.02$). No publication bias was found.

CONCLUSIONS: Specific and overall improvement of HRQoL after BCPS was found. Most of the studies were lacking consistent methodology, had insufficient sample size, and used nonspecific or unvalidated questionnaires. Adopting an accepted, specific, and validated assessment tool for HRQoL in BCPS would improve our ability to assess BCPS outcome.

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Prospective Randomized Control Trial Comparing Electrochemotherapy and Surgery for the Primary Treatment of Basal Cell Carcinoma

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PURPOSE: Basal cell carcinoma (BCC) is the commonest cutaneous malignancy. Surgery remains the gold-standard treatment modality. Electrochemotherapy (ECT), combining electroporation and the administration of normally impermeant chemotherapeutic agents, has demonstrated the efficiency for BCC management but has not been assessed in a prospective randomized setting. This study hypothesized that there would be no difference in response rates between the 2 groups to initial treatment or in treatment durability over time.

METHODS: A prospective randomized control trial was established to determine the efficiency of ECT as a primary treatment modality for BCC with current gold-standard surgery acting as a control. Initial response rates to treatment and recurrence rate during a 3-year period were assessed.

RESULTS: Eighty-six patients with 105 lesions were enrolled and completed minimum follow-up of 1 year (ECT: 45 patients, 60 lesions; surgery: 41 patients, 45 lesions). All patients responded to their primary treatment modality; however, 5 patients in the ECT group required a second ECT treatment and 2 patients in the surgical group required further excision. After 3 years of follow-up, there had been 4 recurrences in the ECT group and 1 recurrence in the surgical group. With loss to follow-up, there is a disease-free progression of 92% (46/50) in those lesions treated with ECT and 97% (32/33) in those treated with surgery ($P = 0.37$).

CONCLUSIONS: Electrochemotherapy is an effective treatment for primary BCC and has a durable effect that is comparable with surgery at 3 years of follow-up.

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Metopic Craniosynostosis: A Demographic Analysis Outside an Urban Environment

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PURPOSE: Metopic craniosynostosis has traditionally been cited as the third most common type of isolated synostosis, after sagittal and coronal craniosynostosis. Recently, several urban institutions have observed an increase in the incidence of metopic synostosis. We sought to determine whether similar demographic changes have occurred in a more suburban setting, and if so, what specific variables were associated with this change.

METHODS: Patients who underwent operative correction of craniosynostosis between 1989 and 2014 were retrospectively reviewed. The type of craniosynostosis as well as gender, family history, birth history, and other demographic data were recorded. Kendall-Mann trend tests and multinomial logistic regressions were conducted, and marginal effects were calculated for all variables included in the model.

RESULTS: Records of 493 patients were reviewed. By using Kendall-Mann trend tests, it was determined that metopic, sagittal, and lambdoid craniosynostoses all demonstrated an increase in incidence. Based on the raw data, metopic synostosis was found to be the second most common type of craniosynostosis between 2004 and 2014. Male gender and multiple gestations were both associated with metopic craniosynostosis.

CONCLUSIONS: This study demonstrated an increasing incidence of metopic craniosynostosis over time, which ascended to the second most common type of synostosis in an analysis outside of an urban environment. In our study, male gender and multiple gestation were positively associated with an increased risk of metopic craniosynostosis. Prospective studies are now needed to further delineate the evolving characteristics of this patient population.

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Defining a New Cell Lineage in the Sagittal Suture and Its Key Pathway

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PURPOSE: Hypoxia signaling pathways are important for bone homeostasis; however, little is known about the role of hypoxia-inducible factor-2 α (HIF-2α) in calvarial development. We have discovered a characteristic phenotype, namely alteration of the sagittal suture as a result of HIF-2α overexpression that has allowed us to define a new cell lineage and the role of HIF-2α in calvarial development.

METHODS: We modulated HIF-2α signaling in vivo within cells of paraxial mesodermal origin. Analysis of mutant (PRX-1Cre; HIF-2α dPAf/+) and control mice (PRX-1Cre; n = 3/time point) was conducted at E12.5, E15.5, P7, P21, and 6 weeks postnatally by micro computed tomography, histology, and whole mount staining. Sagittal suture cells were isolated from 1-week-old HIF-2α dPAf/f mice, transduced with adenovirus encoding β-galactosidase (control) or Cre recombinase (mutants), and evaluated for osteogenic potential.

RESULTS: Confocal microscopy demonstrated recombination at the widened suture, confirming our hypothesis that cells of paraxial mesodermal origin contribute to the sagittal suture and respond to Hif-2α. Bone volume of mutant calvaria was significantly lower than control calvaria ($30.77 \pm 7.21\,mm^3$ vs $55.51 \pm 1.83\,mm^3$, $P = 0.002$). Affymetrix microarray demonstrated that upregulation of Hif-2α caused downregulation of fibroblast growth factor receptor 2.

CONCLUSIONS: We demonstrate that HIF-2α contributes to calvarial and sagittal suture development by modulating cells within the paraxial mesodermal lineage. Defining the role of HIF-2α on calvarial osteogenesis provides knowledge critical to embryonic development.

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Osseodensification: A Novel Approach to Surgical Hardware Fixation

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PURPOSE: Surgical hardware mechanical stability is essential for complex bone reconstruction. Traditional approaches to hardware fixation attempt to maximize stability by interlocking fixation hardware with subtractive drilling of bone. We investigated the effect of additive drilling (osseodensification drilling) in bone hardware fixation from biological and biomechanical perspectives.

METHODS: Four fixation devices were installed on C3 vertebral bodies of 12 sheep; each measuring 4 mm diameter × 10 mm length. The 2 left-sided vertebral body devices were implanted using subtractive drilling, whereas the 2 right-sided devices were implanted using osseodensification. At t = 3 and t = 6 weeks postimplantation, the animals were killed, and the spine segments were retrieved. Identical hardware installation was subsequently performed for C2 and C4, providing fixtures at t = 0. Hardware competence was measured using biomechanical testing of pullout strength, and quality/degree of new bone formation and remodeling was assessed histologically. Statistical analysis included multiple Wilcoxon matched paired tests ($\alpha = 0.05$).

RESULTS: At t = 0, significantly higher ($P = 0.031$ for both C2 and C4) pullout values were observed for osseodensification. Osseodensification also presented significantly higher pullout values at 3 and 6 weeks in vivo ($P = 0.027$). Over time, regular drilling pullout strength increased by approximately 39.5% and osseodensification by approximately 44.2% Histology demonstrated higher degrees of bone surrounding fixtures placed at osseodensification sites relative to regular drilling. Osseodensification bone chips acted as nucleating surfaces for new bone formation.

CONCLUSIONS: Osseodensification increased the degree of biomechanical fixation at time of installation and maintained it as time elapsed in vivo. Histology depicted that osseodensification was not detrimental to bone healing around fixtures.

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Small-Molecule Inhibitor of Bone Morphogenetic Protein Signaling (Idn-193189) Leads to Diminished Bone and Muscle Volume after Burn Plus Tenotomy

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PURPOSE: Bone morphogenetic protein (BMP) receptor inhibitor LDN-193189 has great promise for treatment of genetic and traumatic causes of heterotopic ossification, hereditary hemorrhagic telangiectasia, and fibrodysplasia ossificans progressive. Despite BMP signaling inhibitor's utility, kinase inhibitors can be nonspecific and cause off target effects. In this article, we set out to determine whether therapeutic levels of LDN induces off target effects in a trauma/burn injury model.

METHODS: In vivo studies were performed on C57/BL6 mice using an Achilles' tenotomy with concurrent burn injury model. Micro computed tomography analysis was performed at 5 and 9 weeks after injury to quantify bone and muscle characteristics. The global impact of LDN treatment on SMAD and non-SMAD signaling was assessed using western blot, immunohistochemistry, and immunofluorescence of the hind limb skeletal muscle.

RESULTS: Here, we noted a reduction in bone volume at 9 weeks in the control nontenotomy leg. Similarly, a significant decrease in muscle volume was seen at 5 weeks in the tenotomy leg of LDN-treated mice compared with non-LDN treated mice. The decline in bone and muscle volume correlated with a reduction in P-SMAD 1/5/9, P-ERK 1/2, and VEGF-A and increase in phospho nuclear factor-κB protein expression.

CONCLUSIONS: We demonstrate that BMP receptor kinase inhibitor LDN blocks both SMAD and non-SMAD signaling and shows distinct off target effects on muscle and bone volume. These findings point to potential unwarranted off target effects of using BMP inhibitors after trauma/thermal injury. Clinicians should be aware of these potential side effects when using BMP signaling inhibitors.

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A Retrospective Analysis of Delay to Treatment in Mandibular Fractures and Its Impact on Surgical and Financial Outcomes

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PURPOSE: The aim of this study was to analyze the impact of a delay to treatment of mandibular fractures on surgical outcomes and patient costs and to determine patient characteristics associated with a delay to treatment.

METHODS: Patients who underwent open reduction procedures for mandibular fractures were identified from the 2007 to 2011 Healthcare Cost and Utilization Project California State Inpatient database. Two cohorts were compared: patients who experienced a delay to surgical treatment from admission (2 or more days) and those who did not. Patient demographics, comorbidities, and postoperative outcomes, including complications, readmissions, and length of stay, were compared between groups using multivariable logistic regression.

RESULTS: A total of 6426 patients were included in the study, 19.2% of these cases (n = 1239) demonstrated a delay to surgical repair. These patients were more likely to be older ($P < 0.001$), female ($P < 0.001$), admitted on the weekend ($P < 0.002$), and to suffer from comorbidities including alcohol/drug abuse ($P < 0.001$), chronic hypertension ($P < 0.001$), and chronic lung disease ($P < 0.05$). However, controlling for patient characteristics/comorbidities, a delay to treatment was still shown to significantly contribute to higher complication rates (aOR, 1.84; $P < 0.003$), mortality (aOR, 1.71; $P < 0.001$), and 60-day readmission rates (aOR, 1.60; $P < 0.001$). These patients also experienced an increased length of stay (mean, 9.73 days vs 2.88 days; $P < 0.001$).

CONCLUSIONS: Delay to treatment for mandibular fractures greater than 2 days results in a substantial increase in postoperative complications, readmissions, and hospital stay. These data suggest that reducing the time period between admission and surgery can improve surgical outcomes and reduce complication rates.

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Reduction Gonioplasty: Bone Regeneration and Soft-Tissue Response

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PURPOSE: Reduction gonioplasty is frequently used to achieve an oval-shaped face in Asia. However, the soft-tissue response and bone regeneration of reduction gonioplasty are still unclear. The aim of this study is to evaluate the effects of bone regeneration on facial width and the soft-tissue response of reduction gonioplasty.

METHODS: We retrospectively reviewed patients who underwent reduction gonioplasty from 2009 to 2013. A high-speed rotary cutting bur without a water coolant was routinely used in the gonial region, while the mandibular-chin body osteotomy was performed with a reciprocating saw. Forty-nine patients with preoperative, immediate postoperative, and 12-month postoperative frontal cephalograms were included in our study. Mandible and soft-tissue profiles were measured on cephalograms.

RESULTS: Bone regeneration in high-speed rotary cutting bur osteotomy region was −0.79 ± 1.76 mm (1 cm above Go-Go), −0.75 ± 1.46 mm (Go-Go, bigonial line), −0.77 ± 2.10 mm (1 cm below Go-Go), whereas 0.07 ± 1.79 mm (2 cm below Go-Go) in ostectomy region performed by reciprocating saw. The soft-tissue response ratios were 76.72% ± 30.70% (Go-Go), 108.8% ± 54.11% (1 cm below Go-Go), and 155.9% ± 66.82% (2 cm below Go-Go).

CONCLUSIONS: Bone regeneration does not lead to an increase in facial width after reduction gonioplasty with our technique, and the use of a high-speed rotary cutting bur without a water coolant decreases bone regeneration. The soft-tissue response ratio is higher in the anterior mandible, and the outcome of reduction gonioplasty is a sharper lower face with a full cheek. Reduction gonioplasty is an effective and predictable lower face reshaping surgery.

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Morphometric Evaluation of the Corrugator Migraine Trigger Point

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PURPOSE: Migraine headaches are often attributed to specific peripheral craniofacial trigger points. Some have postulated that hypertrophy of the corrugator muscles causes compression of the supraorbital and supratrochlear nerves and results in migraine headaches. This study uses morphometric evaluation to determine whether anatomic differences exist at the corrugator trigger site between migraine and control patients.

METHODS: A retrospective investigation identified patients with and without migraine headache. Each patient underwent a computed tomography scan for inclusion in the study. By using a three-dimensional image processing program (Vitrea Core), morphometric evaluation of the corrugator muscles was performed in a randomized and blinded fashion on 90 migraine headache and 90 control patients. Measurements were also normalized to zygoma-to-zygoma distance to account for variations in skull size. When sidedness of the symptomatic corrugator trigger was documented, further subgroup analysis was conducted. Statistical comparisons were performed using *t* tests.

RESULTS: Among migraine patients, the mean corrugator volume was 0.97 ± 0.25 cm^3 compared with 1.12 ± 0.30 cm^3 in controls (P = 0.0003), whereas the mean maximum thickness was 5.35 ± 0.89 mm compared to 5.61 ± 0.93 mm in controls (P = 0.054). In subgroup analysis of 28 patients, mean anatomic measurements from the symptomatic side were compared with those of the mean contralateral asymptomatic side. The mean symptomatic corrugator volume was 0.95 ± 0.18 cm^3 versus 0.98 ± 0.15 cm^3 (P = 0.60). The mean symptomatic corrugator thickness was 5.25 ± 0.87 mm versus 5.40 ± 1.01 mm (P = 0.54).

CONCLUSIONS: Muscle hypertrophy in itself does not play a major role in triggering migraine headaches. Instead, factors such as muscle hyperactivity or peripheral nerve sensitization may be more causative.

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Autogenous Reconstruction of Large Secondary Skull Defects

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PURPOSE: As defect sizes increase, skull reconstructions become more challenging, forcing many to use alloplasts. We sought to ascertain the upper limits of autogenous reconstructions and examine outcomes compared with published data on synthetic reconstructions.

METHODS: A retrospective review of all autogenously reconstructed critical-sized secondary skull defects was undertaken. A literature search (Cochrane databases, Ovid, and PubMed) was performed using the key words: "cranioplasty," "skull defect," and "calvarial defect," with <10 patients and <1-year follow-up as exclusion criteria.

RESULTS: Over 14 years, 133 patients were reconstructed exclusively with autogenous bone and 96 had complete records. The mean reconstructive age was 12.9 years (1–65 years), and the defect size averaged 93 cm^2; 23% had extremely large defects, between 120 and 506 cm^2. Thirty percent had undergone previous reconstruction elsewhere using alloplasts. Operative time averaged 3.4 hours; transfusion rates, 2%; hospital length of stay, 3 days; and follow-up, 23 months (maximum: 19.6 years). There were no postoperative infections. One patient (1%) experienced a partial resorption and underwent a secondary cranioplasty. Twenty-seven studies (1954 patients) met our inclusion criteria for a meta-analysis. Compared with published alloplastic reconstructions, infection and other complication rates were significantly lower for this autogenous series: 5.7% versus 0% ($P < 0.016$) and 9.4% versus 2% ($P < 0.015$), respectively.

CONCLUSIONS: Although many surgeons faced with large secondary skull defects might be compelled to use alloplasts, autogenous reconstructions are technically feasible with skull defect sizes as large as 500 cm^2. Compared with alloplasts, autogenous reconstructions have significantly lower long-term infection and complication rates.

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Time Interval Reduction for Delayed Implant-Based Cranioplasty in the Setting of Previous Bone Flap Osteomyelitis

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PURPOSE: Reinfections after implant-based cranioplasty, in the setting of previous bone flap osteomyelitis, are common and associated with significant morbidity. Currently, the timing of reconstruction after initial osteomyelitic bone-flap removal remains controversial; most advocate for prolonged time intervals around 6 to 12 months. With this in mind, we chose to investigate our delayed cranioplasty outcomes after both "early" (between 90 and 179 days) and "late" (≥180 days) time intervals with custom cranial implants to determine whether timing affected outcomes and rates of reinfection.

METHODS: A retrospective cohort review of 25 consecutive cranioplasties performed at a multidisciplinary center from 2012 to 2014 was conducted under institutional review board approval. A nonparametric bivariate analysis compared variables and complications between the 2 different time interval groups defined as "early" cranioplasty (between 90 and 179 days) and "late/delayed" cranioplasty (≥180 days).

RESULTS: No significant differences were found in primary and secondary outcomes in patients who underwent "early" versus "late" delayed cranioplasty ($P > 0.29$). The overall reinfection rate was only 4% (1/25), with the single reinfection occurring in the "late" group. Overall, the major complication rate was 8% (2/25). Complete and subgroup analyses of specific complications yielded no significant differences between the early and late time intervals ($P > 0.44$).

CONCLUSIONS: Results suggest that "early" delayed cranioplasty is a viable treatment option for patients with previous bone flap osteomyelitis and subsequent removal. As such, a reduced time interval of 3 months—with equivalent outcomes and reinfection rates—represents a promising area for future study and aims to reduce the morbidity surrounding prolonged time intervals.

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Repeal of Universal Helmet Laws: Do Motorcycle Helmet Laws Affect the Incidence of Craniomaxillofacial Trauma?

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PURPOSE: Motorcycle helmet legislation has been a contentious topic of debate for over a half-century. On April 13, 2012, the state of Michigan repealed a 35-year-old universal motorcycle helmet law in favor of a partial helmet law. Loss of motorcycle tourism dollars was cited as the major motivating factor. We describe the early clinical effects on craniomaxillofacial trauma and mortality at a level 1 trauma center in West Michigan.

METHODS: Motorcycle trauma patients presenting to a level 1 trauma center 3 years before and after the law repeal (n = 534) were included in a retrospective cohort study.

RESULTS: After repeal, the rates of nonhelmeted trauma patients increased from 7% to 30%. Overall rates of facial fractures increased from 13.2% to 14.6%, although this did not reach statistical significance. Crash-scene mortality of nonhelmeted patients increased dramatically from 6.7% to 61%, and motorcyclists admitted to the hospital were over twice as likely to require ventilator support. No difference was observed with respect to total length of stay, intensive care unit length of stay, Injury Severity Score, or Glasgow Coma Scale. Compared with helmeted motorcyclists, nonhelmeted patients were twice as likely to sustain facial fractures and had double the rate of hospital mortality. Patients with facial fractures were nearly 5 times more likely to die after admission compared with those without.

CONCLUSIONS: This study highlights the significant negative impact of relaxed motorcycle helmet laws. Although craniomaxillofacial trauma trended toward a statistical significant increase, helmet use and crash-scene mortality dramatically increased.

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Connective Tissue Growth Factor Ablation Impairs Palatal Mesenchyme Growth

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PURPOSE: Nonsyndromic cleft palate is the most common craniofacial birth defect. Despite its high incidence, our understanding of its etiology remains poor. To date, multiple mouse models (transforming growth factor-β-3 KO, IRF-6 KO) have been utilized but have been limited by varying penetrance and severity. We have previously identified CTGF KO mice as a novel model to study cleft palate development.

METHODS: CTGF KO and WT palates were isolated and grown in explant cultures. Histologic analyses were performed on explants at various time points. Next, mesenchymal cells were isolated from CTGF KO and WT palates and compared for cellular organization, proliferation, and migration. mRNA from palatal tissues was isolated and analyzed for alterations in known palatogenesis signaling pathways.

RESULTS: Palatal explant cultures demonstrated that CTGF KO palates fail to grow horizontally and fuse compared with WT shelves. In addition, when artificially approximated in culture, CTGF KO palates remain clefted. Mesenchymal cells isolated from palatal shelves demonstrate decreased proliferation and altered migration, adhesion, and spreading. Abrogation in bone morphogenetic protein, transforming growth factor-β, FGF, and Wnt signaling pathways are seen in CTGF KO cells.

CONCLUSIONS: Defects in palatal mesenchyme growth contribute to the cleft palate phenotype of CTGF KO mice. Multiple signaling pathways necessary for normal palate development are affected by CTGF ablation. Our explant model is being used to elucidate the key mechanism(s) responsible for cleft palate in CTGF KO mice. These studies will further our understanding of cleft etiology and may elucidate novel therapeutic targets for clinical management of this birth defect.

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Higher Dosages of BMP-2 Result in Higher Rates of Postoperative Nasal Stenosis When Used in Alveolar Cleft Repair

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PURPOSE: A former surgeon at our institution frequently used bone morphogenetic protein-2 (BMP-2) instead of autograft for alveolar bone reconstruction (ABR). Patients with BMP-2 ABR appeared to have higher rates of postoperative nasal stenosis. Different dosages of BMP-2 were compared to determine whether there was a dose-dependent relationship.

METHODS: We retrospectively reviewed cleft patients who underwent ABR. We excluded patients with preexisting nasal stenosis. Six concentrations of BMP-2 were used in the alveolar cleft (milligrams per milliliter): 0, 1.05, 2.1, 4.2, 8.4, and 12.

RESULTS: Sixty patients underwent 115 surgeries meeting criteria: surgeries involving BMP-2 (BY), 48% and no BMP-2, 52%. Postoperative nasal stenosis was BY (62%), no BMP-2 (30%), $P < 0.001$. However, BY were more likely to involve concurrent nasal repairs $P < 0.001$. Logistic regression using the predictor variables BMP-2 status, concurrent nasal repair status, showed that only BMP-2 status was predictive for postoperative nasal stenosis: odds ratio, 3.49 (95% confidence interval, 1.06–11.46) $P = 0.04$. When BMP-2 concentration was used as a predictor variable with 6 ordinal levels in a logistic regression that adjusted for concurrent nasal surgery, BMP-2 dose level is a dose-dependent predictor of postoperative nasal stenosis: odds ratio, 1.244 (95% confidence interval, 1.030–1.503), $P = 0.023$.

CONCLUSIONS: In patients receiving BMP-2 during alveolar cleft repair, higher rates of postoperative nasal stenosis were observed as greater doses of BMP-2 were used.

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Investigation of the Use of Bone Marrow Stem Cells to Facilitate Pathologic Fracture Healing of the Mandible After Irradiation

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PURPOSE: Radiation therapy for head and neck cancer results in devastating morbidity for bone healing including the complications of osteoradionecrosis and subsequent pathologic fractures. Our investigation seeks to improve bone healing through the use of bone marrow stem cells (BMSCs) in a mandibular osteotomy defect after radiotherapy. Our hypothesis was that use of BMSCs would improve union rates and bone mineralization metrics thereby improving clinical outcomes

METHODS: Lewis rats (n = 31) were divided into 3 groups: nonradiated fracture (Fx), radiated fracture (XFx), and radiated fracture with BMSCs (XFxBMSC). The groups receiving radiation were administered a human-equivalent dose of 35 Gy over 5 days. Two weeks later, the rats received a mandibular osteotomy and external fixation to 2.1 mm. The animals were killed at postoperative day 40, their mandibles were harvested, examined for bony union, and micro computed tomography scan was performed. Metrics of bone mineral content, bone mineral density, tissue mineral content, tissue mineral density, and bone volume fraction were ascertained.

RESULTS: Although only 20% of the XFx group demonstrated unions, 66% of the XFxBMSC group had unions. For all mineralization metrics, a significant decrease was observed between Fx and XFx, and a significant remediation was imparted with BMSC therapy. Even more impressive was that no statistically significant differences were seen between the Fx and XFxBMSC groups.

CONCLUSIONS: BMSCs significantly improve the clinically relevant metrics of bony union and mineralization in a model of irradiated mandibular fracture healing. Given our results, we are proponents of additional studies to translate this promising therapy to clinical investigation.

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Electronic Cigarettes Are as Toxic to Skin Flap Survival as Tobacco Cigarettes

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PURPOSE: Electronic cigarettes (e-cigarettes) have become increasingly popular. However, information about the health risks associated with vaping is sparse. There are currently no published studies examining the effects of e-cigarettes on microcirculation or perfusion. By using a rat skin flap model, we examined the toxic effects on microcirculation and perfusion e-cigarettes may have in comparison with tobacco cigarettes.

METHODS: Sixty rats were divided into a room air group, a tobacco cigarette smoke exposure group, a medium-nicotine content (1.2%) e-cigarette vapor exposure group, and a group exposed to a high-nicotine content (2.4%) e-cigarette vapor. After 20 days of exposure, a random pattern, 3 × 9-cm skin flap was elevated on the dorsum of the rats. At 25 days of exposure, flap survival was evaluated digitally, and the rats were killed. Plasma was collected for nicotine and cotinine analysis, and flap tissues were harvested for histopathological and biochemical assays.

RESULTS: Digital evaluation of the dorsal skin flaps demonstrated significantly increased necrosis in the vapor and tobacco groups. The average necrosis within the groups was as follows: control, 18.0%; high-dose vapor, 28.6%; medium-dose vapor, 35.9%; and tobacco cigarette, 30.1%. Although the e-cigarette and tobacco cigarette groups did not differ significantly, each individual group had significantly more necrosis than the control group ($P < 0.05$).

CONCLUSIONS: Both the medium- and high-nicotine content e-cigarette exposure groups have similar amounts of flap necrosis when compared with the tobacco cigarette exposure group. Nicotine-containing e-cigarette vapor may be just as toxic to skin flap survival as tobacco cigarettes.

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Settling the Controversy: Lymph Node Transfer or Multiple Lympho-Venous Anastomoses? A Prospective Long-Term Case-Control Experimental Study in Pigs

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PURPOSE: The best surgical approach for limb lymphedema is highly debated, with long-term histological evidence still lacking to support either the transfer of lymph nodes (LNT) or performing multiple lympho-venous anastomoses (MLVA) as the best solution. The purpose of this study is to objectively quantify and compare LNT and MLVA efficiency in improving lymphatic flow in a pig experimental model.

METHODS: A prospective experimental study was designed using 14 common breed pigs (*Sus scrofa domesticus*), with surgically induced lymphedema, which were divided into 4 groups: LNT heterotopic transfer (n = 4), MLVA (n = 4), LNT + MLVA orthotopic transfer (n = 4), and control group (n = 2). Indocyanine green (ICG) near-infrared fluorescence imaging and Dark Blood MR lymphangiography with dual-agent relaxivity contrast were used to assess the preoperative and the 5 months postoperative lymphatic status. Histological analysis was performed using monoclonal D2-40 antibody and hematoxylin and eosin staining.

RESULTS: Both ICG and dual-agent relaxivity contrast successfully identified new lymphatic vessels, allowing for objective quantification of lymphangiogenesis, confirmed by the histological findings. At 5 months after surgery, LNT showed a 33% increase in lymphangiogenesis compared with MLVA, and LNT + MLVA showed a 62% increase compared with the control group.

CONCLUSIONS: Although MLVA provide superficial lymphatic return flow, LNTs generate a regional response with lymphangiogenesis and improved superficial and deep lymphatic drainage. Ideally, where ICG imaging indicates a benefit from MLVA, surgeons should combine both techniques to maximize lymphatic flow.

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Oxygen Sensing Liquid Bandage: A Novel Approach in Tissue Perfusion Assessment

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PURPOSE: Oxygenation status and early detection of vascular compromise are fundamental in the prevention of postoperative flap failure after microsurgical breast reconstruction. Various noninvasive and invasive modalities have emerged as an adjunct to standard clinical monitoring; however, no current method is ideal. In this study, we propose a novel, noninvasive approach that allows for assessment of tissue perfusion by using a newly developed oxygen sensing paint-on dye incorporated into commercially available liquid bandage matrix material.

METHODS: A thin film of porphyrin-based, oxygen sensing dye was applied to the skin surface of 8 rats. The infrarenal aorta was dissected and clamped at 1-minute intervals for 20 minutes and subsequently unclamped. Signal intensity was captured by a camera-based imaging device to create a 2-dimensional mapping of skin tissue oxygenation. Near infrared imaging and a Clark electrode were used as control methods.

RESULTS: Clamping the aorta resulted in a rapid decrease in oxygenation. More importantly, registered oxygenation demonstrated similar pattern among groups along with recovery.

CONCLUSIONS: This proof-of-concept study demonstrates the capacity of oxygen sensing films to accurately detect underlying tissue oxygenation and provides a basis for further development of noninvasive tissue oxygenation assessment.

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In Vivo Anastomosis of Tissue-Engineered Vascular Network

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PURPOSE: An obstacle in modern tissue engineering is the inability to create constructs with their own inherent vascular networks. Such an experimental model would be a step toward the construction of clinically applicable artificial tissue for transplantation. Previously, we fabricated a cellularized, biocompatible, vascular construct with smooth muscle and endothelial lining comparable with native vessels. Herein, a biocompatible vascular construct was microsurgically anastomosed to rat native vessels.

METHODS: Two 2-mm diameter by 7-mm length channels made of Gore-Tex were bridged by a U-shaped loop made of Pluronic F127 macrofiber, which sacrificed in 1% type I collagen. A Sprague Dawley rat right femoral artery and vein were isolated, heparinized (100 units/1 ml saline), and anastomosed to the construct fabric catheters using 9-0 sutures.

RESULTS: Anastomosis of the tissue engineered vascular construct was successful in vivo. The fabric catheters were well integrated in the collagen scaffold, and the sacrificial channel was patent. The construct maintained its structure throughout the length of the surgery, and continuous flow from the arterial to the venous side of the construct was observed.

CONCLUSIONS: We successfully fabricated and anastomosed a biocompatible, vascularized tissue-engineered construct. In addition to studying its microvascular architecture and its response to various stimuli in vivo, this prototype may be developed to recapitulate full-thickness skin flaps. Our innovative, tissue-engineered construct holds tremendous promise for the future of microsurgery and artificial tissue transplantation.

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Topical Vasodilator Mimics Surgical Delay to Improve Cutaneous Flap Viability and Induce Vascular Remodeling

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PURPOSE: Surgical delay is a well-known technique that improves perfusion of random and pedicle cutaneous flaps. The aim of this study was to create a model of pharmacological delay that would induce vascular remodeling and decrease overall flap necrosis.

METHODS: A modified caudally based McFarlane flap was created using a rat model. Seven groups of random flaps were created (n = 8) that included application of topical minoxidil and iloprost for various durations beginning 2 weeks before flap elevation. A standard 2-week surgical delay group was performed for a positive control. Surgical flaps were elevated, reinserted, and observed at various time points until postoperative day 7. Gross viability, histology, perfusion analysis, tissue oxygenation, and vascular casting were analyzed.

RESULTS: Pharmacologic delay with preoperative application of topical minoxidil or iloprost was found to have equivalent flap viability when compared with standard surgical delay. A significant increase in viability was observed when comparing these groups with a negative control using topical vehicle alone. Pharmacologic delay was found to increase blood flow during the preoperative period through vascular remodeling. These changes were not observed in flaps that were only treated in the postoperative period.

CONCLUSIONS: Preoperative topical application of vasodilators yielded equivalent increases in viability in a random cutaneous flap model compared with standard surgical delay. This improvement in tissue viability using topical vasodilators that are approved for human use may reduce potential for postoperative complications without additional surgical procedure.

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The Versatility of the Medial Femoral Condyle Flap: 1 Surgeon's Experience

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PURPOSE: The medial femoral condyle (MFC) flap has arguably become the flap of choice for treatment of small to medium size critical bony defects. This article aims to describe the versatility of the MFC in treating a variety of bony defects.

METHODS: A retrospective chart review was performed from 2009 to 2014 on all patients who underwent free MFC flaps.

RESULTS: Twenty-two patients were identified: 17 upper and 6 lower extremity bony reconstructions. The upper extremities included 7 scaphoid nonunions, 3 lunate avascular necrosis, 2 ulnar nonunion, 2 proximal humerus reconstructions, and 2 metacarpal nonunions, and 1 clavicle. The lower extremity reconstructions included 5 for talus and 1 for navicular. The average was 44 years (18–64 years) and average body mass index was $29.3 \pm 6.59\,kg/m^2$. In 15 cases, hardware was used to stabilize the bone flap, and in the remaining cases, suture or impaction was used to immobilize the flap. Average follow-up was 28.4 ± 23.24 months. Ultimately, 77% (17 of 22) demonstrated complete or partial radiologic bony union. Of the 5 who had nonunion, 2 were symptomatically improved. Notably amongst scaphoid patients union was 86%. A significant correlation was found between higher body mass index and nonunion ($P < 0.05$).

CONCLUSIONS: For these complex bone defects, we have obtained a satisfactory union rate despite a morbid patient population. The MFC flap has the advantages of a reliable pedicle, optional skin paddle, and minimal donor morbidity.

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Free Tissue Transfer for Lower Extremity Reconstruction in Thrombophilic Patients: A Comparison of Prophylactic Anticoagulation Protocols

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PURPOSE: Subclinical-thrombophilia contributes to delayed thrombotic complications/nonsalvageability after free tissue transfer (FTT). Nevertheless, there is no consensus regarding optimal perioperative anticoagulation in these patients. We present our experience with lower extremity FTT in thrombophilic patients and compare outcomes for subcutaneous versus systemic anticoagulation protocols.

METHODS: Between 2012 and 2015, 52 patients with subclinical-thrombophilia underwent FTT for nontraumatic, lower extremity reconstruction. Patients were stratified into 2 main cohorts based on whether subcutaneous or systemic heparin was administered. Beginning day 0, patients were given daily aspirin (325 mg) and either (1) subcutaneous heparin (5000 units every 8 hours), (2a) fixed rate subtherapeutic heparin infusion (500 units/h), or (2b) titrated therapeutic heparin infusion (PTT 50–60). In the absence of complications, patients were converted to subcutaneous heparin on day 5 and continued on this regimen for a period of 3 weeks. Demographic data, reconstructive outcomes, and complications were retrospectively compared.

RESULTS: Twenty-seven patients were treated with aspirin/subcutaneous heparin, whereas 25 patients received aspirin/systemic heparinization with either fixed rate (n = 10) or titrated (n = 15) heparin infusion. Flap loss (20% vs 4%, $P = 0.066$) and rates of thrombosis (16% vs 7%, $P = 0.41$) were higher among patients who received systemic heparinization. On subgroup analysis, failure rates were higher (26% vs 4%, $P = 0.027$) after titrated systemic heparinization when compared with subcutaneous administration. Bleeding complications were also more prevalent among these patients when compared with those in both fixed rate (13% vs 0%) and subcutaneous (13% vs 11%) cohorts ($P = 0.22$ and 0.045, respectively).

CONCLUSIONS: Prophylactic anticoagulation with aspirin/subcutaneous heparin is both safe and effective in thrombophilic patients who undergo lower extremity FTT. The risks of bleeding complications and thrombosis associated with systemic heparin do not justify its routine use in this population.

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Laser Speckle Contrast Imaging Is a Promising Tool for Monitoring of Partial and Full Venous Outflow Obstruction—An Experimental Study in a Porcine Flap Model

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PURPOSE: In microsurgery, there is a demand for more reliable methods for flap monitoring. We used laser speckle contrast imaging and laser Doppler flowmetry to assess partial and full venous outflow obstruction in a porcine flap model.

METHODS: Cranial gluteal artery perforator flaps were raised, and arterial and venous blood flow was monitored using ultrasonic flow probes. The venous flow was altered with an inflatable cuff to simulate partial and full (50% and 100%) venous obstruction. The flap microcirculation was monitored using laser speckle contrast imaging (LSCI) and laser Doppler flowmetry (LDF).

RESULTS: After partial (50%) venous occlusion, perfusion decreased from baseline (LSCI: $P = 0.007$, LDF: $P = 0.07$). After 100% venous occlusion, a further decrease in perfusion was observed (LSCI: $P = 0.01$, LDF: $P = 0.05$). After release of the venous cuff, LSCI detected a return of the perfusion to a level slightly below baseline level ($P = 0.001$), whereas the LDF signal returned to a level not significant from baseline ($P = 0.99$). During 50% and 100% venous occlusion, LSCI showed a 20% and 26% intersubject variability (CV %), respectively, compared with 50% and 77% for LDF.

CONCLUSIONS: LSCI offers sensitive and reproducible measurements of flap microcirculation and seems more reliable in detecting decreases in blood perfusion caused by venous obstruction. Also, it allows for perfusion measurements in a large area of flap tissue. This may be useful in identifying areas of the flap with compromised microcirculation during and after surgery.

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Eulerian Video Magnification for Flap Monitoring: Touch Free Perfusion Visualization

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PURPOSE: The gold standard for microsurgical free flap monitoring is clinical evaluation, frequently with the assistance of a pencil or surface Doppler device. However, such techniques depend largely on the skill and experience of the operator. Furthermore, although several technologies have been developed to monitor flap perfusion with varying degrees of sensitivity and specificity, they are frequently cumbersome, expensive, and invasive. Here, we introduce a novel, digital video-based flap monitoring system that is noninvasive, operator independent, and economical as a way of evaluating flap perfusion.

METHODS: Digital videos of rat abdominal fasciocutaneous flaps and human free flaps, finger replants, and perforator flaps were obtained and augmented using Eulerian video magnification open-source software provided by the Computer Science and Artificial Intelligence Laboratory at MIT.

RESULTS: Eulerian video magnification applied to the flap videos resulted in an enhanced video clip, which amplified subtle variations in perfusion and allowed users to "see" perfusion throughout the flap. Heart rate and perfusion quality were assessed in the videos and corroborated with clinical evaluation of flap health and physiology.

CONCLUSIONS: Here, we demonstrate proof of concept in both animal and human models that a novel noninvasive flap monitoring technology can reliably evaluate flap perfusion. The technology is free, sensitive, and simple to use. We anticipate that further development will result in an end-user device application (eg, mobile app), which can evaluate flap viability in a safe, effective, and inexpensive fashion.

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Fluorescein Isothiocyanate: A Novel Application for Intraoperative Lymphatic Imaging

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PURPOSE: The LYMPHA technique entails performing a lymphovenous bypass at the time of axillary lymph node dissection to reduce lymphedema risk. We developed a novel application of fluorescein isothiocyanate (FITC) for intraoperative lymphatic imaging. Our goal is to demonstrate safety and efficacy of FITC for this application.

METHODS: We reviewed a prospectively collected database on breast cancer patients who underwent LYMPHA from March to September 2015. FITC was utilized to identify arm lymphatic channels after axillary lymph node dissection to perform a lymphovenous bypass between disrupted lymphatics and axillary vein tributaries. Data on preoperative and intraoperative variables were analyzed.

RESULTS: Thirteen patients underwent LYMPHA with intraoperative FITC lymphatic imaging from March to September 2015. Average patient age was 50 years with a mean body mass index of 28 kg/m^2. On average, 3.4 lacerated lymphatic channels were identified and 1.7 channels were bypassed per patient. Eleven anastomoses were performed to the accessory branch of the axillary vein and 1 to a lateral branch. In 1 patient, a bypass was not performed because of poor lymphatic caliber and inadequate length of vein tributary. No intraoperative adverse events were noted.

CONCLUSIONS: FITC is a safe and effective method for intraoperative lymphatic imaging. FITC imaging maintains life-like color in nonfluoresced tissues allowing for simultaneous dissection and lymphatic visualization. Moreover, blue dye can now be reserved for use by our oncologic surgeons.

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Fluorescein Isothiocyanate: A Modification for Reverse Lymphatic Mapping in Lymph Node Transplantation

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PURPOSE: Reverse lymphatic mapping (RLM) reduces the risk of donor-site lymphedema during lymph node transplantation (LNT) but is limited as mapping and dissection cannot be performed simultaneously. We hypothesized that RLM with fluorescein isothiocyanate (FITC) would provide this advantage. Our goal was to demonstrate the safety and efficacy of FITC for RLM.

METHODS: We reviewed a prospectively collected database of patients undergoing LNT utilizing our modified RLM technique from March to September 2015. FITC was injected into the donor extremity, and flap dissection was performed under a Yellow 560 filter to simultaneously visualize and avoid critical donor lymphatic channels.

RESULTS: Twelve patients underwent LNT utilizing our modified RLM technique from March to September 2015. Average patient age was 59 years with a mean body mass index of 35 kg/m^2. Eleven superficial circumflex iliac artery flaps and 1 transverse cervical flap were harvested. In 4 cases, FITC lymphatic imaging improved precision of the dissection plane. No intraoperative adverse events were noted. Donor-site complications included 3 seromas and 1 cellulitis. Postoperative donor extremity lymphatic evaluations were normal in all patients at an average of 6.7 months, except for 1 patient diagnosed with an ipsilateral DVT at 3 months.

CONCLUSIONS: Our modified RLM technique with FITC is a safe modification adding precision to RLM. FITC maintains life-like color in nonfluoresced tissues allowing for simultaneous dissection and lymphatic mapping.

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Implantable Optical Oxygen Monitor to Diagnose Flap Compromise

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PURPOSE: Surgical flaps experience perfusion compromise in the immediate postoperative period. Early identification and correction of ischemia improves salvage. Current oxygen monitoring techniques are difficult. We developed a novel approach to monitor tissue oxygenation using implantable optical sensors and correlated with the loss of flap viability.

METHODS: Sensors were made by incorporating benzoporphyrin dye into poly(2-hydroxy ethylmethacrylate). Sensors were approximately 3 mm long, 1.5 mm wide, and 0.5 mm thick. Male Sprague-Dawley rats had the planned skin flap outlined on rat dorsum, and 3 sensors were intradermally implanted at tip, middle, and base of impending flap. Three sensors were implanted as controls laterally. Inspired O_2 was modulated from 100% to 12%. One day later, flap was elevated. Gross flap viability was assessed with planimetric analysis. Readings from sensors were obtained by measuring decay rate of phosphorescence after transdermal excitation of O_2-sensitive fluorophore on days 0, 3, and 7 postoperatively. Sodium fluorescein was injected to identify perfusion.

RESULTS: Oxygen readings by sensors were modulated as expected when inspired oxygen changed, confirming sensors responsiveness/sensitivity. Gross analysis showed approximately 16% necrosis at the tip of flap on day 3 and was more pronounced on day 7. Sodium fluorescein analysis showed progressively decreased perfusion in tip of flap, becoming significantly evident on day 7($^*P < 0.05$) with approximately 70% flap viability. Readings from flap sensors showed significant decreases in oxygenation in all regions at all time points compared with control sensors. Further regional analysis showed that sensors detected significant decrease in oxygenation in tip of flap in comparison with base at all time points ($^*P < 0.05$).

CONCLUSIONS: Flap oxygenation was assessed using novel sensors. Our sensors were able to detect significant decreases in oxygenation immediately after creating flap. Regional analysis showed that the decrease was more pronounced at the tip of flap where necrosis later developed, making continuous oxygen measurement more sensitive in predicting flap viability.

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A 1-Year Comparison of Outcomes for Normal Saline and an Antiseptic Solution for Negative Pressure Wound Therapy with Instillation

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PURPOSE: Negative pressure wound therapy with instillation is an adjunctive treatment to debridement that utilizes periodic installation of a solution and negative pressure. The objective of this study was to compare normal saline versus an antiseptic solution that is more often used.

METHODS: This randomized, prospective longitudinal effectiveness study compared 0.9% normal saline versus 0.1% polyhexanide + 0.1% betaine for adjunctive treatment of infected wounds requiring hospitalization and operative debridements. Number of operative visits, length of stay, and proportion of closed, infected, or dehisced wounds at several time points were measured.

RESULTS: Of the 94 patients randomized, there was no statistically significant difference between the demographic profiles between the 2 cohorts except for a larger proportion of males to females ($P = 0.004$). Percentage of closed wounds at 30, 60, 90, 180, and 360 days after discharge for normal saline and antiseptic solution were 66.7% and 65.2%, 56.1% and 45.5%, 73.7% and 54.8%, 61.3% and 50%, 78.6% and 66.7%, with drop out of 0, 20, 25, 37, and 45, respectively. Of the time points recorded, none showed a statistical significant difference in wound closure.

CONCLUSIONS: For patients treated with negative pressure wound therapy with instillation as an adjunct to debridement, there was statistically no difference in wound closure over 1 year, length of index hospitalization, or number of operations before 30 days between use of compared 0.9% normal saline versus 0.1% polyhexanide + 0.1% betaine. Antiseptic solutions are commonly used and are associated with significant cost, based on these findings; normal saline is equally efficacious and can be used as a substitute.

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Topical JAG1, a Notch Activator, Accelerates Closure of Splinted Cutaneous Excisional Wounds in Wild-Type Mice

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PURPOSE: Annually in the United States, >6.5M patients suffer from wound-related complications, and treatment cost is >$25B. We are interested in improving the wound healing process. We have previously shown that inhibition of Notch decreases wound healing. Therefore, in this experiment, we hypothesize that activation of the Notch pathway, via JAG1, would accelerate the rate of wound healing in a splinted cutaneous excisional wound model.

METHODS: Eight-week old, male mice (n = 44) were anesthetized, shaved, and two 1 cm^2 full-thickness wounds were created on their backs under aseptic conditions. A 12-mm stent was secured around each wound to prevent healing by contraction. Sterile dressings were applied. Dressings were changed daily after topical application of JAG1 (10 nM) or vehicle (PBS) for 17 days. Digital photographs were taken daily. Wounds were analyzed using ImageJ. Wound closure was defined by visualization of resurfacing epithelia. Statistical significance was defined as $P < 0.05$ using the Students' *t* test.

RESULTS: Wound sizes were similar in both groups at the beginning of treatment. JAG1 significantly accelerated the rate of wound closure compared with control by day 11 (11.18 ± 1.15 vs 7.59 ± 1.13 respectively, $P < 0.03$) and remained significantly accelerated throughout the experiment. Wound closure for the JAG1 group occurred on day 17. The control group was not completely closed at day 17.

CONCLUSIONS: JAG1, a notch activator, accelerates cutaneous wound closure in vivo. Based on this novel finding, further studies should evaluate the mechanisms by which Notch accelerates wound healing. This could result in the development of new therapeutics for wound healing in patients.

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Systemic Antioxidant Therapy Enhances Regenerative Capacity of Diabetic Skin

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PURPOSE: Diabetes-associated chronic nonhealing wounds impose an overwhelming burden, where measures like glycemic control are insufficient to promote closure. We previously showed that the cytoprotective pathway, Nrf2-Keap1, is dysregulated in diabetes. When we restored antioxidant production, diabetic wound healing rates were accelerated. Here, we investigated whether oral Nrf2 induction (NI) therapy promotes diabetic wound closure.

METHODS: By using 10-week-old Lepr-db/db mice, we made 10-mm diameter excisional wounds in dorsal skin. We gavaged the mice daily with vehicle, low-dose (LD) NI, or high-dose NI. We assessed wound time to closure, histological analysis, and molecular analysis of 10-day-old wounds.

RESULTS: LD-NI oral therapy reduced closure time to 23.3 days, versus 30 days with vehicle therapy, $P = 0.0017$. High-dose -NI-treated wounds heal in 26.83 days, nonsignificant versus vehicle control. LD-NI decreased pathologic healing time by 42%, vs untreated wounds and vehicle control, and decreased wound burden by 29%, $P < 0.05$, vs untreated wounds, and by 42% compared with vehicle-treated wounds, $P < 0.05$. LD-NI therapy resulted in the fastest wound healing rate (log rank $P = 0.0008$). Hematoxylin and eosin stains on 10-day wound sections showed over 2-fold decrease in epithelial gap with LD-NI therapy, versus vehicle therapy, $P < 0.05$. Granulation tissue area and $CD31^+$ vasculature increased 5-fold and 1.5-fold, respectively with LD-NI therapy. The antioxidant NQO1, downstream of Nrf2, was upregulated >3× with LD-NI therapy, vs vehicle therapy.

CONCLUSIONS: Cutaneous wound closure in diabetic mice is significantly reduced with oral LD-NI therapy. LD-NI improves molecular and cellular composition of wound beds by upregulating Nrf2-mediated antioxidants. The data support advancement of NI as a therapeutic modality for chronic diabetic wounds.

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Topical Minocycline Effectively Decontaminates and Reduces Inflammation in Porcine Wounds

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PURPOSE: Topical antibiotics have potential to treat wound infection and inflammation while minimizing adverse effects associated with systemic antibiotics.

METHODS: Full-thickness wounds were created on 4 pigs and then randomized for *Staphylococcus aureus* infection. By using polyurethane wound enclosure devices, wounds were treated with 100 μg/mL minocycline, 1000 μg/mL minocycline, or saline control. Bacteria were quantified in wound tissue and fluid obtained over 9 hours. Immunosorbent assays were used to analyze wound fluid concentrations of interleukin (IL)-1β, IL-6, tumor necrosis factor-α, and matrix metalloproteinase-9. Inflammatory cells were quantified in wound tissue using immunohistochemistry.

RESULTS: After 6 hours, 100 and 1000 μg/mL minocycline decreased bacteria in wound tissue to 3.5 ± 0.87 and 2.9 ± 2.3 log colony-forming unit/g, respectively, compared with 8.3 ± 0.9 log colony-forming unit/g in control wounds ($P < 0.001$). After 2 hours, minocycline reduced inflammatory cytokines IL-1β, IL-6, and tumor necrosis factor-α concentrations ($P < 0.01$). Wound tissue inflammatory cell counts decreased after 1 hour ($P < 0.05$). In noninfected wounds, minocycline significantly reduced IL-1β, IL-6, and inflammatory cell counts after 4 hours ($P < 0.01$). Matrix metalloproteinase-9 concentrations decreased after 1-hour treatment ($P < 0.05$).

CONCLUSIONS: Topical minocycline rapidly decontaminates infected wounds while significantly reducing local inflammation. The ability of minocycline to reduce inflammation exists even independent of its antibacterial effect, suggesting its potential to improve wound healing and reduce scarring.

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Single-Cell Analytics Identify Novel Subpopulation of Cells with Enhanced Wound Healing

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PURPOSE: Mesenchymal stem cells hold great promise in regenerative medicine. These cells are thought to function through a combination of (1) secretion of progenitor-recruiting trophic factors, (2) modulation of the local immune response, (3) enhancement of angiogenesis, and (4) improvement of extracellular matrix production. These effects represent a comprehensive collection of the various therapeutic approaches to wound healing. A caveat in their safe clinical use is the incomplete understanding of their mechanism of action, made difficult by cellular heterogeneity. We aim to utilize bioinformatics approaches to characterize functionally distinct subsets of human bone marrow-derived mesenchymal stem cells (BMMSC) and determine the mechanism that underlies their efficacy.

METHODS: Passage 3 human BMMSCs were subjected to high-throughput single-cell multiplex quantitative polymerase chain reaction, looking at 96 manually curated genes pertaining to wound healing. Advanced mathematical modeling was applied to this multidimensional data to unravel heterogeneity and decipher cell surface markers that will enable functional testing. Elucidated subpopulations were subjected to fluorescence activated cell sorting for functional testing in diabetic (DB/DB mice).

RESULTS: Single-cell analysis revealed 2 transcriptionally distinct subpopulations with different protein signatures (subpopulation 1: vasculogenic vs subpopulation 2: immunomodulatory and remodeling). Functional tests of these subpopulations in diabetic mice revealed accelerated wound closure by subpopulation 2 (DB/DB: unsorted: 26.5 ± 0.95 days, subpopulation 1: 25.5 ± 0.73 days; $P = 0.4$; subpopulation 2: 20.1 ± 1.12 days, $P = 0.0008$).

CONCLUSIONS: We demonstrate the utility of bioinformatics in unraveling BMMSC heterogeneity to identify a subset of BMMSCs that heal diabetic wounds 6 days faster than heterogeneous BMMSCs.

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Transitioning from Scarless Fetal Wound Healing: Uncovering the Responsible Fibroblast Lineage

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PURPOSE: Early in utero cutaneous wounds heal without a scar. Many studies have offered possible explanations for this phenomenon but no definitive answer has emerged. We have previously characterized a scar-forming fibroblast lineage in the dorsal skin of adult mice defined by embryonic expression of Engrailed-1 (En1). Here, we investigate the role of this lineage during fetal wound healing.

METHODS: En1-derived fibroblasts were traced by crossing En1-Cre and ROSA26-mTmG mice. A murine model of fetal scarless wound healing allowed for investigation of En1-derived fibroblast behavior before and after the scarless transition. En1-derived fibroblasts were characterized using flow cytometry and RNA sequencing analysis at various stages of embryonic development.

RESULTS: Dorsal wounds created at embryonic day 16.5 (e16.5) healed scarlessly with minimal connective tissue deposition. However, wounds created at e18.5 healed with substantial scar deposited primarily by En1-derived fibroblasts. The relative number of En1-derived fibroblasts and the expression of CD26, a previously identified marker of the En1 lineage, steadily increased from e12.5 through postnatal day 1. RNA sequencing analysis of En1-derived fibroblasts revealed that e18.5 fibroblasts express a highly fibrogenic program in comparison with e16.5 fibroblasts in unwounded skin ($*P < 0.01$).

CONCLUSIONS: The En1 lineage of fibroblasts plays a critical role in the transition from scarless wound healing during fetal development. These results hold promise for the development of therapeutic approaches to fibrotic disease and adult wound healing.

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Nrf2 Deficiency Delays Diabetic Wound Healing

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PURPOSE: Redox homeostasis management is critical in cutaneous wound healing, especially in a diabetic environment. We found that upregulating Nrf2-mediated antioxidants in diabetic wounds promotes reepithelialization with well-vascularized granulation tissue. We aimed to elucidate whether Nrf2 signaling in the epidermis and vasculature are impaired in diabetic skin.

METHODS: Five-micrometer tissue sections from 10-mm diameter excisional humanized cutaneous wounds on wild-type (WT), diabetic, and Nrf2−/− mice were stained for K14, K10, CD31, and Nrf2 using immunofluorescence to visualize spatial expression.

RESULTS: Nrf2 is highly expressed in basal K14+ epidermis, K10+ cells, and $CD31^+$ endothelial cells in WT skin. Nrf2 is upregulated in epidermis, dermis, and granulation tissue by 7 days postwounding with gradient expression longitudinally with higher signal nearby wound bed. Nrf2 shows gradient expression in dermis and lower signal in deep dermis. Nrf2 is downregulated in intact diabetic skin epidermis, dermis, and fails to upregulate after wounding. Compared with WT, Nrf2−/− wounds generate 17% less granulation tissue (223,500 $units^2$ versus 305,900 $unit^2$), have 29% lower concentration of $CD31^+$ cells(3.0 vs 4.3 cells/hpf) in granulation tissue at 7 days after wounding, and demonstrate 24% longer time to closure (17.3 vs 14.0 days), $P < 0.05$.

CONCLUSIONS: Nrf2 expression is highly expressed in tissues that are known to cope with high levels of ROS. Similarities between Nrf2−/− and diabetic skin wounds suggest that decreased Nrf2 expression is a driving factor for poor diabetic wound healing because of poor ROS management in epidermis, granulation tissue, and endothelial cells. Our results validate the targeting of Nrf2/Keap1 pathway to develop effective therapies for diabetic wounds.

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Adipose-Derived Stem Cells Promote Wound Healing of Irradiated Abdominal Muscle in a Rat Model

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PURPOSE: Owing to their adult stem cell characteristics and easy isolation with conventional liposuction procedures, adipose-derived stem cells (ASCs)-based treatments have been expanded substantially in recent years. However, the therapeutic effect of ASCs treatment on irradiated muscle tissue is still poorly understood. This study aimed to determine whether ASCs could facilitate wound healing of irradiated abdominal muscle in a rat model.

METHODS: Aged Fischer rats with varied dose irradiation on abdomen underwent laparotomy based on our established protocol. Rats were divided randomly into 6 groups according to the radiological dose and with or without intramuscular injections of ASCs isolated from healthy rat. Fourteen days postoperatively, muscle samples were harvested and divided into 3 pieces for mechanical tests, histological and immunohistochemical staining, and protein array analysis.

RESULTS: Mechanical tests showed that mechanical properties of abdominal muscle were impaired by radiation and enhanced by ASCs treatment. Elastic modulus (0.88 ± 0.35 MPa) and ultimate tensile strength (0.35 ± 0.10 KPa) of group 4 were significantly strong than group 3 ($P < 0.05$). Hematoxylin and eosin staining indicated that ASCs mitigated inflammatory cell infiltration by migration and localization to the wound interphase. A higher expression of CD31 in endothelial cells and increased capillary density were observed in ASCs-treated rats ($P < 0.05$), which suggested that ASCs stimulated new blood vessel formation. Masson Trichrome and MyoD1 staining showed that ASCs treatment improved collagen deposition and induced muscle regeneration ($P < 0.05$).

CONCLUSIONS: ASCs accelerate wound healing of irradiated abdominal muscle via their capability to modulate inflammation, induce angiogenesis, and activate myogenesis.

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Gastrin-Releasing Peptide and Scleroderma: A Potential Novel Mechanism of Tissue Fibrosis

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PURPOSE: Scleroderma (SSc), chronic collagen vascular disease, affects >100,000 Americans yearly. Early, patients develop contractures and intradermal fibrosis, and death occurs from multiple-organ involvement. There is no treatment for SSc. Recently, mouse model of SSc was reported. Two laboratories showed that reactive oxygen species (ROS) cause this dermal fibrosis. We observed that gastrin-releasing peptide (GRP) mediates pulmonary fibrotic responses to other ROS sources: hyperoxia or radiation. We hypothesize that ROS mediate dermal fibrosis via GRP release from cutaneous nerves during ischemia-reperfusion injury similar to SSc. GRP-positive sensory nerves do mediate neurogenic inflammation.

METHODS: Ten-week-old C3H/HeJ female mice were injected intradermally on the back with bleomycin (100 μg/d) for 3 weeks. Immediately after bleomycin, some mice were given potent antioxidant *N*-acetylcysteine intraperitoneally. Other bleomycin-injected mice were given GRP blockade intraperitoneally with monoclonal antibody 2A11 twice weekly. After 3 weeks, skin specimens were processed for routine histopathology, including Masson's trichrome staining. Dermal thickness was quantified by an observer (E.M.) with no knowledge of mouse identities, comparing manual ruler measurements with ImageJ.

RESULTS: Bleomycin mouse model was validated by using dermal thickness as a measure of fibrosis, as widely described. Bleomycin alone resulted in 39% increase in dermal thickness, which was completely inhibited by *N*-acetylcysteine. In 1 experiment, mice given bleomycin+2A11 for GRP blockade had approximately 50% reduction in dermal thickness with minimal collagen deposition compared with bleomycin alone.

CONCLUSIONS: Our study confirms ROS trigger bleomycin-induced dermal fibrosis. This ROS effect appears to be mediated by GRP. Ongoing studies evaluate vasculature, nerves, macrophages, markers of ROS and hypoxia, and GRP-related genes. This could clarify how dermal fibrosis could result from other ROS sources including radiation, burns, or graft-versus-host disease. New concepts of SSc pathogenesis could lead to novel therapeutic approaches.

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Dermal Biological Scaffold and Regenerate for Traumatic Wound Coverage—A Case Series of 240 Wounds

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PURPOSE: Bioartificial dermal regeneration templates (DRTs) have been used extensively in burn treatment, but few studies have reported their use in combat wounds. Combat injuries represent devastating wounds that require restoration of the protective skin barrier while preserving function of exposed muscle, tendons, and nerves. This study reports the impact and outcomes of DRTs in the reconstruction of traumatic combat extremity wounds.

METHODS: An institutional review board-approved retrospective review of patients treated with Integra DRT (Integra Lifesciences Corporation, Plainsboro, N.J.) for combat-related wounds from 2009 through 2013 was completed. The primary outcome investigated was healing of wounds after DRT placement, as measured by successful take or stable coverage with skin grafting, flap procedure, or in the cases of delayed primary closure, healing of the closed wound.

RESULTS: One hundred ninety patients with 280 wounds met inclusion criteria, of whom 251(90%) had complete records. Patients underwent a median of 3 irrigation and debridement procedures before DRT placement during a median of 8 days. The median time from DRT placement to definitive closure was 15 days. The median time from injury to definitive closure was 35 days. Overall healing rate after first attempt definitive closure was 86%.

CONCLUSIONS: Bioartificial DRTs have played an increasing role in the treatment of traumatic war wounds. By utilizing the biologic scaffold, our clinical group has been able to successfully achieve definitive closure of wounds complicated by avascular tissue while maintaining adequate function. This study reports the largest consecutive case series of DRTs employed for traumatic injuries in the literature.

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Aerobic Exercise Improves Obesity-Induced Lymphatic Dysfunction Independent of Weight Loss

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PURPOSE: Obesity has been shown to decrease lymphatic function and, in turn, amplify the pathologic responses in obesity. However, the cellular mechanisms that regulate this response remain unknown. The purpose of this study was to analyze lymphatic vascular changes in obese mice and determine whether these pathologic effects are reversible with aerobic exercise.

METHODS: Male C57/BL6J mice were fed a high-fat diet for 10 to 12 weeks to induce obesity and then were randomized to an aerobic exercise group (treadmill running for 6 weeks) or a sedentary group that was not exercised. Lean control mice remained sedentary and fed a normal chow diet. At the conclusion of the experiment, we analyzed lymphatic function, histological changes, and *lymphatic endothelial cell* (*LEC*) gene expression.

RESULTS: Sedentary obese mice had markedly decreased collecting lymphatic vessel pumping capacity, decreased lymphatic vessel density, decreased lymphatic migration of immune cells, and increased lymphatic vessel leakiness compared with lean animals. Aerobic exercise, independent of weight loss, markedly improved lymphatic function and significantly decreased perilymphatic accumulation of inflammatory cells and inducible nitric oxide synthase expression, compared with sedentary obese mice. In addition, exercise normalized isolated *LEC* gene expression of lymphatic specific genes (*VEGFR-3* and *Prox1*).

CONCLUSIONS: This study, for the first time, suggests that implementation of aerobic exercise in the setting of obesity can improve lymphatic function and may limit some of the pathologic consequences of obesity, independent of weight loss. These findings provide a mechanism for recommending aerobic exercise to patients who are at risk for developing lymphedema.

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Inhibition of Fibrosis Decreases the Pathology of Lymphedema

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PURPOSE: Lymphedema is characterized by fibrosis, and there is accumulating evidence that this process plays a key role in the pathology of the disease. We have previously shown that the expression of the profibrotic growth factor transforming growth factor (TGF)-β is increased in lymphedematous tissues. The purpose of this study was to evaluate the efficacy of this topical compound for prevention and treatment of lymphedema.

METHODS: We developed a novel topical antifibrotic compound and tested its efficacy using mouse tail and popliteal lymphatic ablation models. Control animals were treated with vehicle only. Adipose deposition, lymphatic function, perilymphatic inflammation, and lymphangiogenesis were analyzed. In addition, to test the hypothesis that this topical formulation can treat established lymphedema, we analyzed the efficacy of this treatment using a transgenic model of lymphedema 6 months after lymphedema was established.

RESULTS: Our topical anti-TGF-β compound markedly decreased fibrosis, swelling, and adipose deposition. These changes correlated with improved lymphatic transport function compared with controls ($P < 0.001$). Similarly, transgenic mice with established lymphedema also had decreased fibrosis, adipose deposition, and perilymphatic inflammation when compared with controls. Furthermore, this treatment resulted in significantly decreased perilymphatic T-cell inflammation ($P < 0.005$) and markedly increased lymphangiogenesis as assessed by near infrared imaging and histology.

CONCLUSIONS: Treatment with a topical anti-TGF-β compound markedly decreased fibrosis and chronic T-cell inflammation and improved lymphangiogenesis in various models of lymphedema and lymphatic dysfunction. These results are exciting as they suggest that topical treatments may be useful clinically.

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Proposed Pathway and Mechanism of Vascularized Lymph Node Flaps

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PURPOSE: Microsurgical treatment of lymphedema with vascularized lymph node (LN) transfer can improve signs and symptoms of disease, but the pathways and mechanisms of these flaps warrant further exploration. The objective of this study is to investigate the pump mechanism and pathway of lymph transit in vascularized LN flaps.

METHODS: Animal model: 72 flaps were raised in 18 rats: 36 groin flaps contained LNs, and 36 deep inferior epigastric artery perforator flaps did not (non-LN). Indocyanine green (ICG) was added into normal saline (NS) and 1%, 3%, 5%, 7%, and 10% albumin. LN and non-LN flaps were submerged in solution and surveyed for venous fluorescence. In the 7% albumin and NS groups, volumetric change of solution was measured. Human model: A similar experiment was performed in humans using 5 submental LN flaps.

RESULTS: Animal model: Fluorescence was detected in the venous pedicle of LN flaps submerged in 5%, 7%, and 10% albumin, and half of flaps submerged in 3% albumin. Fluorescence was not detected in LN node flaps submerged in ICG-containing NS or 1% albumin solution. Fluorescence was not detected in non-LN flaps. There was greater volume reduction with LN flaps than non-LN flaps ($P < 0.001$). Human model: Fluorescence was detected in the venous pedicle of all flaps immersed in lymph.

CONCLUSIONS: ICG fluorescence was detected in the venous pedicle of rat and human LN flaps submerged in lymph or albumin when the concentration was greater than 3%. Based on these results, a pathway for lymphatic uptake is presented.

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Preoperative Paravertebral Block Improves Health Resource Utilization and Reduces Postoperative Pain in Patients Undergoing Autologous Breast Reconstruction after Mastectomy for Breast Cancer

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PURPOSE: There is increasing evidence that preoperative paravertebral blocks (PVBs) reduce postoperative pain and length of stay (LOS) for patients undergoing surgical treatment of breast cancer, including mastectomy with prosthetic reconstruction. However, evidence on the efficacy of PVB in autologous breast reconstruction is lacking. The purpose of this study is to determine whether preoperative PVB impacts postoperative pain control and LOS in patients undergoing autologous breast reconstruction.

METHODS: We performed a retrospective matched case-control analysis of consecutive patients undergoing postmastectomy autologous breast reconstruction from 2012 to 2014 comparing those who received PVB with those who did not. Primary outcomes included self-reported pain score, time to oral-only narcotic usage (TTON), and LOS. Sample differences were compared using Wilcoxon rank sum and chi-square tests for continuous and categorical variables, respectively. Kaplan-Meier analysis was used to evaluate TTON and LOS, with Mantel-Cox test used to compare groups.

RESULTS: Of 78 patients, 39 received PVB and 39 did not. Cases and controls did not differ regarding age, body mass index, American Society of Anesthesiologists class, exposure to chemotherapy or radiation, mastectomy type, flap type, unilateral versus bilateral surgery, or cancer stage ($P > 0.05$). PVB patients reported significantly lower postoperative pain at 2 and 24 hours ($P < 0.01$) and exhibited significantly shorter median TTON (66 vs 76 hours, $P < 0.01$). Importantly, median LOS was significantly reduced for PVB patients in both hours (95 vs 116, $P < 0.01$) and hospital nights (4 vs 5, $P = 0.05$).

CONCLUSIONS: This is the first study to demonstrate that preoperative PVB significantly reduces LOS and postoperative pain in patients undergoing autologous breast reconstruction.

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Comparing the Psychological and Cosmetic Outcomes of Nipple-Sparing Mastectomy with Simple Mastectomy

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PURPOSE: Reconstruction of the nipple-areolar complex has been shown to be a crucial factor in a woman's satisfaction and psychological well-being postmastectomy. Nipple-sparing mastectomy (NSM) preserves the natural appearance of the breast and may preserve nipple sensation and erectile function. The purpose of this pilot study was to investigate the influence that NSM has on women's body image and sexuality and to assess the level of satisfaction with breast reconstruction.

METHODS: Questionnaires adapted from the validated Sexual Adjustment and Body Image survey were sent out to 30 simple mastectomy patients and 42 NSM patients who had undergone mastectomy at Stony Brook University Hospital between 2010 and 2014.

RESULTS: Worsened body image was reported in 53.3% of patients who had NSM compared with 29.4% of patients who had simple mastectomy. Negative effects on sexual satisfaction and sexual relationships were reported in more NSM patients than simple mastectomy patients. After reconstruction, 40% of NSM patients reported being satisfied with nipple sensation and 66.7% of NSM patients with erectile function of the nipple. Average satisfaction with nipple appearance and position was greater in NSM patients than in simple mastectomy patients.

CONCLUSIONS: The preliminary data suggest that although patients undergoing NSM have a higher level of satisfaction with aesthetic outcomes of breast reconstruction, they may also experience more negative effects on body image and sexuality than patients undergoing simple mastectomy. Patients undergoing NSM should be extensively counseled on the possible detrimental effects that they may experience as a result of mastectomy and breast reconstruction.

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Dual Venous Outflow Associated with Improved Outcomes in Lower Extremity Trauma Free Flap Reconstruction

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PURPOSE: Venous outflow problems are the most common reasons for perioperative flap complications. The benefit of a second venous anastomosis, however, remains unclear in lower extremity trauma free flap reconstruction and warrants further investigation.

METHODS: Retrospective review of our institutional flap registry from 1979 to 2014 identified 464 free flaps performed for lower leg trauma reconstruction. Patient demographics, flap characteristics, and outcomes were examined.

RESULTS: Two hundred nineteen flaps were randomly selected for preliminary analysis. Single-vein outflow was more common (72.6%) than dual-vein outflow (27.3%); majority of recipients were in deep venous system (83.5%) versus superficial (11.9%) or both (4.6%). Fasciocutaneous flaps were more likely to have 2 veins than muscle flaps ($P = 0.002$). Complications occurred in 96 flaps (43.8%), with 54 partial flap losses (24.7%) and 11 complete flap losses (5.0%). Dual venous outflow was associated with significant reduction in overall complication rate (RR = 0.37, $P = 0.003$). Multivariable regression analysis controlling for age, sex, flap type, flap size, vein size mismatch, and time since injury demonstrated dual-vein outflow to be protective against partial flap failure (RR = 0.30, $P = 0.040$) and any flap failure (RR = 0.26, $P = 0.011$). No significant difference in operative time was found ($P = 0.664$).

CONCLUSIONS: Dual-vein outflow demonstrated 63% reduction in overall complications and 74% reduction in flap failure rate compared with single-vein flaps. These results suggest a protective effect of a dual-vein outflow system, and when considered together with our findings of unchanged operative time, provide evidence for preferential use of 2 venous anastomoses when possible for free flap reconstruction of lower extremity trauma.

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The Underlying Reasons and Timing for Readmission in Plastic Surgery Procedures

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PURPOSE: Risk factors for readmission are well known in plastic surgery, but the underlying reasons and timing of these events have yet to be investigated.

METHODS: Data from the National Surgical Quality Improvement Program (2012 to 2013) regarding the underlying reasons for readmission in plastic surgery procedures were consolidated into 17 categories. The incidence, timing, and reasons for readmission were analyzed for the overall cohort and common, individual procedures. Kaplan-Meier survival curves were developed for readmission events, and median days to readmission were calculated for each underlying reason.

RESULTS: Overall, 1267 (3.8%) of 33,021 patients were readmitted. Of these, 17.7% (n = 206) were unrelated to the index operation. Surgical site infection (SSI) was the most common reason for readmission (n = 310, 26.7%), followed by hematoma/hemorrhage (n = 101, 8.7%), prosthesis-related complications (n = 77, 6.6%), surgical complications (n = 72, 6.2%), and wound disruption (n = 71, 6.1%). Reasons varied by procedure type, but SSI was consistently the most frequent reason. Readmission risk is high immediately after discharge and progressively tapers by 30 days. The most common early drivers of readmission included hemorrhage, hematoma, and gastrointestinal complications; late drivers were SSI, sepsis, and wound disruption.

CONCLUSIONS: Readmissions in plastic surgery can be largely attributed to new complications arising after discharge from the index hospitalization. The underlying reasons for readmission vary by procedure in a time-sensitive manner and should be considered in future policy targeting readmission reduction.

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Effect of Postoperative Complications on Patient Satisfaction: Results from a Cohort of 200 Breast Reconstruction Patients using Breast-Q and RAND-36

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PURPOSE: Despite advances in surgical techniques and quality improvement, considerable numbers of breast reconstruction patients still experience postoperative complications. This study investigated the effect of postoperative complications on breast specific and general quality of life in patients who underwent breast reconstruction.

METHODS: We analyzed prospectively collected patient-reported quality of life measurements on all patients undergoing breast reconstruction at our institution between November 2010 and June 2013. Breast-Q and RAND-36 scores were available preoperatively, after tissue expander placement, and 6 and 12 months after final reconstruction. We used *t* tests and rank sum tests to assess the effect of postoperative complications on patient satisfaction and quality of life.

RESULTS: Of 200 patients completing long-term follow-up, 32 (16%) experienced postoperative complications. Patients with complications had significantly decreased Breast-Q scores for satisfaction with information (12.74 points; 95% confidence interval, 1.34–24.14; $P = 0.029$) and satisfaction with outcome (17.90 points; 95% confidence interval, 4.12–31.68; $P = 0.012$). These differences were also clinically relevant. We found suggestive evidence for a decrease in satisfaction with surgeon ($P = 0.053$) and satisfaction with office staff ($P = 0.050$) and no evidence for a difference in RAND-36 Physical ($P = 0.442$) and Mental ($P = 0.281$) Summary Scores.

CONCLUSIONS: These findings highlight the negative effects of postoperative complications on patient satisfaction, which go beyond well-characterized clinical outcomes and economic concerns. In particularly, our results imply the importance of patient safety interventions and educating patients preoperatively to mitigate lower satisfaction and QOL scores.

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Soft Tissue Reconstruction of Large Spinal Defects: A 12-Year Institutional Experience

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PURPOSE: Radical spinal resections can lead to large soft tissue defects requiring reconstruction. Instrumentation, irradiated tissue, and patient comorbidities all increase wound complication risk. The purpose of this study was to review our experience with soft tissue spinal reconstruction and identify risk factors predictive of wound complications.

METHODS: We retrospectively reviewed patients who underwent spinal resection and required soft tissue reconstruction from 2002 to 2014. Patient conditions, defect location, indication/method of reconstruction, and wound complication and reoperation rates were collected. Logistic regression was performed to determine risk factors for wound complications.

RESULTS: Of 289 reconstructions performed in 259 patients, 224 were performed prophylactically at time of spinal resection in anticipation of wound complications, and 65 were performed therapeutically for postoperative wound complications. Locoregional paraspinous muscle flaps were most commonly used. The major wound complication rate (ie, requiring operative intervention) was similar between prophylactic and therapeutic indications (24.1% vs 15.4%, $P = 0.136$). Patients with prophylactic reconstructions had lower mortality (0.9% vs 9.2%, $P < 0.001$) and instrumentation removal rates (0.9% vs 4.6%, $P = 0.043$). On logistic regression, presence of instrumentation [odds ratio (OR), 3.7; $P = 0.006$], requirement of free flap (OR, 11.3; $P = 0.041$), and age of 40 to 54 years (OR, 2.5; $P = 0.048$) were associated with increased major wound complications.

CONCLUSIONS: Spinal resections carry significant surgical site morbidity, and selection of high-risk patients for prophylactic reconstruction with locoregional flaps may decrease wound complication rates.

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A Simple, Prognostic Severity Scale for Unilateral Cleft Lip

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PURPOSE: There is no universally accepted severity scale for unilateral cleft lip that objectively quantifies the spectrum of disease, making it difficult to evaluate postoperative outcomes in the context of preoperative severity.

METHODS: Anthropometric measurements and photographs were prospectively collected from unilateral cleft lip patients from Morocco, Bolivia, Vietnam, and Madagascar during medical missions. The following were obtained preoperatively and postoperatively: columellar angle, cleft width, nostril width, vertical lip height, and horizontal vermillion length. The number of primary cleft lip repairs done in his/her lifetime was recorded for each surgeon. Our previous study showed vertical lip height symmetry (cupid's bow to subalare) best predicts how surgeons and lay-persons rank surgical outcomes. Therefore, we defined "unacceptable" postoperative outcome/symmetry as cleft side/noncleft side vertical lip height discrepancy >3 mm, as studies show that human eyes detect down to 3 mm of asymmetry in the nasolabial region.

RESULTS: Of the 149 patients included, 22 had unacceptable outcomes. Multivariate and stepwise models showed preoperative cleft width ratio (CWR; preoperative cleft width divided by commissure width) was the most significant predictor for unacceptable outcomes, controlling for surgeon experience. CWR was normally distributed. Two severity categories were created: grade 1: CWR < 5, grade-2: CWR ≥ 0.5. Grade 2 patients had a higher likelihood of unacceptable outcomes (odds ratio, 2.9, 95% confidence interval, 1.1–7.7, $P = 0.029$). The risk of having unacceptable outcomes was higher for grade 2 (27%) versus grade 1 (11%). The probability of having acceptable outcomes for grade 2 individuals was lower versus grade 1 (PPV = 73% vs 89%).

CONCLUSIONS: Our scale utilizes a simple, intuitive ratio to classify patients into clinically prognostic categories.

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Can Patient-Reported Outcomes Measurement Information System Capture Health-Related Quality of Life among Children with Cleft Lip and Palate?

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PURPOSE: Accurate measures of health-related quality of life among children with cleft lip and palate (CLCP) are in high demand. The National Institutes of Health-validated Patient-Reported Outcomes Measurement Information System (PROMIS) could provide data to compare outcomes across conditions, but the accuracy and feasibility of these measures remain unknown.

METHODS: We surveyed children younger than 5 years with CLCP from a multidisciplinary clinic (n = 93). Children completed PROMIS Anxiety, Depression, and Peer relationship item banks by short form or computerized adaptive testing (CAT) and the Pediatric Quality of Life Inventory for comparison. Construct validity was measured by Spearman's correlation coefficients. Feasibility was defined by instrument completion time and reading level. Multivariate regression analyses controlled for race, gender, age, and income.

RESULTS: PROMIS was significantly correlated with Pediatric Quality of Life Inventory scores (PROMIS: Peer Relationships: r = 0.50, $P < 0.001$; Anxiety : r = −0.42, $P < 0.001$; Depression: r = −0.50, $P < 0.001$), with easier readability. Compared with short form versions, CAT administration demonstrated minimal floor (0% vs 0%) and ceiling (8.6%–17.5% vs 21.8%–41.9%) effects. Although CAT templates took significantly longer to complete (Anxiety: 63.4 ± 36.8 vs 31.9 ± 13.1 seconds, $P = 0.002$; Depression: 62.7 ± 42.8 vs 31.7 ± 22.1 seconds, $P = 0.01$; Peer relationships: 75.3 ± 55.3 vs 50.7 ± 39.1 seconds, $P = 0.13$), children answered more questions within this time.

CONCLUSIONS: PROMIS demonstrates similar accuracy, with greater sensitivity and readability compared with existing measures of health-related quality of life among children with CLCP. Use of such instruments will improve our ability to compare children with CLCP to diverse populations and clinical conditions in a longitudinal fashion.

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National and Regional Trends in Autologous Breast Reconstruction

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PURPOSE: The incidence of breast cancer (BC) cases has increased significantly over the past decades. Hence, breast reconstructive procedures follow this trend constituted both by autologous and implant-based reconstructions. The aim of this study was to assess national and regional trends in different types of autologous breast reconstruction (ABR).

METHODS: By using the Nationwide Inpatient database (2008–2012), data on BC and mastectomy rates, type of ABR, and sociodemographics were obtained and analyzed. Furthermore, national and regional trends over time for ABR were plotted and analyzed.

RESULTS: A total of 427,272 patients were diagnosed with BC, of whom 343,165 underwent mastectomy and 152,256 received BR of which 16.4% had ABR. Overall, ABR demonstrated a significant increase over time (6.4%–17.6% between 2008 and 2012, $P < 0.001$), but when stratified per region, this positive trend was only seen in the Midwest and the Southern region. Most ABR over time was performed in the Southern region (37.4%). Subgroup analysis demonstrated a significant increasing trend for both LD and DIEP flap, both nationally and regionally. Interestingly, most pTRAM, fTRAM, SIEA, and GAP flaps were performed in the Northeast region, whereas most DIEP and LD flaps were performed in the Southern region.

CONCLUSIONS: Overall, autologous breast reconstruction showed a significantly positive trend over time, but when stratified into region, this was only seen in the Midwest and the Southern regions.

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Trends in Nonvascularized Bone Grafting for Nonunion of the Scaphoid

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PURPOSE: The scaphoid is the most commonly fractured bone of the carpus. Because of its retrograde blood supply, fractures of the scaphoid are predisposed to nonunion. Untreated, scaphoid nonunion progresses to carpal collapse and radiocarpal arthrosis. Nonvascularized bone grafting is commonly employed to reconstitute the defect. Incidence and outcomes of this procedure are not well described.

METHODS: The PearlDiver Patient Records Database was queried using *International Classification of Diseases*, Ninth Revision codes to identify patients with scaphoid nonunion from 2007 to 2013. Current Procedural Terminology codes were then used to isolate patients treated with nonvascularized grafting.

RESULTS: Nonunion of the scaphoid was identified in 20,788 patients, and the annual incidence increased in a linear fashion ($P < 0.001$). Of this cohort, 1.67% (n = 347) were treated with nonvascularized bone grafting. The rate of this procedure decreased during the study time period ($P < 0.001$); however, reimbursement increased ($P < 0.05$). Incidence of scaphoid nonunion was highest in adults aged 60 to 79 years, whereas nonvascularized grafting was performed most often in patients aged 10 to 29 years. Males were more likely to undergo this procedure than females ($P < 0.001$).

CONCLUSIONS: Incidence of scaphoid nonunion is increasing; however, nonvascularized bone grafting is being utilized less frequently to address this well-recognized complication. Nonunion is more common in the elderly, but nonvascularized grafting is performed more often in younger patients.

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The Largest Series of Gastrocnemius Muscle Flaps for Salvage of Total Knee Arthroplasty

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PURPOSE: Total knee arthroplasty (TKA) surgery, while generally successful, can develop devastating surgical site complications. The gastrocnemius muscle flap is the gold standard for TKA salvage. Its utility is incompletely understood because of limited data from case reports and patient series. We present the largest experience of TKA salvage using the gastrocnemius flap.

METHODS: A retrospective review of all patients undergoing gastrocnemius flap for TKA salvage between 1998 and 2014 was performed in accordance with the University of Pittsburgh Institutional Review Board. Indications included exposed hardware, infection, fistula, wound dehiscence, and nonhealing wound. Preoperative risk factors were identified. Rates of postoperative complications, need for revision, explantation, and amputation, as well as ultimate ambulation status, were analyzed.

RESULTS: Sixty-nine patients were identified: male, 43%; average body mass index, 31.4 kg/m^2; smoker, 35%; diabetes, 32%. Primary indication for TKA was arthritis (64%). Primary indication for gastrocnemius flap was open wound (51%). Fifty-four patients had >1 year of follow-up (average 3.5 years). Of those, 33% ambulated independently, 43% ambulated with assist, 13% were nonambulatory, and 11% required amputation. Patients were less likely to independently ambulate if obese ($P = 0.02$) or if their indication for TKA was arthritis ($P = 0.01$). Smokers were more likely to develop flap-related complications ($P = 0.01$).

CONCLUSIONS: Patients undergoing gastrocnemius muscle flap for TKA salvage showed durable long-term wound stability. The gastrocnemius flap is useful in allowing patients with threatened TKAs achieve ambulation, but still has a significant salvage failure rate. These data will aid in counseling patients undergoing attempted TKA salvage.

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Identification of a Circulating Pericyte that Homes To Ischemia

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PURPOSE: Pericytes are nonhematopoietic cells found adjacent to endothelial cells in blood vessels. They assist in neovascularization by scaffolding and stabilizing endothelial cells. However, the origin, identity, and fate of pericytes remain unclear. Here, we test for a circulating pericyte after ischemic injury.

METHODS: Murine parabiosis is established between a fluorescent reporter mouse (donor) and a nonfluorescent syngenic mouse (recipient). Hindlimb ischemia or ischemic flaps are created on the recipient mouse after establishment of cross-circulation. The ischemic sites on the recipient mouse are isolated 7, 14, 28, or 180 days after injury. All circulating nonimmune cells are isolated by fluorescent-assisted cell sorting and subjected to single-cell transcriptional analysis for genes specific to stemness, neovascularization, and growth factor receptors to determine the fate and profile of cells. Bone marrow of the reporter mouse is transplanted into nonfluorescent syngenic recipients, and ischemic flaps are made on the recipients to determine the source of the circulating cells.

RESULTS: Single-cell analysis of nonimmune cells recruited to ischemia identifies a unique bone marrow-derived pericyte that attaches to endothelial cells at the site of injury, deposits extracellular matrix, and proliferates after engraftment. We find no circulating cell that differentiates into an endothelial cell at the site of ischemia.

CONCLUSIONS: By using lineage tracing and single-cell analytics in mice, we show the existence of a unique bone marrow-derived circulating pericyte that homes to ischemia. Our findings have implications in aging, tumorigenesis, and disease.

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In Vitro and In vivo Interaction of Adipose-Derived Stem Cells and Breast Cancer Cells: Is Fat Grafting Safe in Postmastectomy Patients?

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PURPOSE: We investigate the in vitro and in vivo interaction of breast cancer cells (BCCs) and adipose-derived stem cells (ASCs) in an attempt to better understand the clinical risks of fat grafting in postmastectomy breast reconstruction.

METHODS: For in vitro study, BCCs and ASCs were obtained from the same patient. A homogenous ($CD90^{-}/CD24^{+}$) BCC population was obtained with flowcytometric cell sorting. The effect of ASCs on migration of BCCs was examined using a cell migration assay. In vivo arm of the study was performed using MDA-MB-231 BCCs and patient-derived ASCs/fat grafts. BCCs were injected to the fourth mammary gland of female nude mice (n = 20) bilaterally. A total of 65×105 BCCs, 1.45×105 ASCs, and 150 µl of unprocessed fat graft were injected in corresponding groups. Tumors were followed up with serial digital caliper measurements and examined histologically after 4 weeks.

RESULTS: The percentage of $CD90^{-}/CD24^{+}$ BCCs in initial cell population was 0.61%. BCCs migrated approximately 10-folds more when cocultured with ASCs compared with BCC-only cultures ($P < 0.01$). Tumor growth rate in group III and group IV was significantly higher than group I ($P < 0.01$). Histologically, injected fat grafts were largely replaced by BCCs after 4 weeks.

CONCLUSIONS: ASCs significantly increase the in vitro migration of BCCs in cocultures and in vivo growth of breast cancer xenografts.

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Stem Cell Subpopulation Profiling as a Predictor of Human Health and Regenerative Capacity

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PURPOSE: Adipose-derived stem cells (ASCs) are essential for tissue homeostasis and readily accessible for therapeutic purposes. Autologous therapy outcomes are variable despite refinements to harvest, processing, and application techniques. We examined whether comorbidity-related depletion of ASC subpopulations could contribute to poor therapeutic responses and morbidity by examining ASCs at a single-cell level.

METHODS: The stromal vascular fraction was isolated from humans (n = 110) and mice (n = 40) across a wide spectrum of age and comorbidities. Magnetic-assisted cell sorting achieved lineage depletion before single-cell sorting ($CD45^{-}CD31^{-}CD34^{+}$) by fluorescence-activated cell sorting. Gene expression profiles were determined using 96 genes across a spectrum of stemness and related genes. Subpopulations were resolved by a fuzzy-C means cluster-based algorithm and then verified by surface marker profiling using density-dependent (ViSNE) and independent (SPADE) analyses. Subpopulation profiles were correlated with 360 clinical variables during a median follow-up of more than 500 days.

RESULTS: Animal models of diabetes mellitus, obesity, and aging demonstrated depletion of ASC subpopulations important for neovascularization—a novel cellular basis for the complication profiles of these states. These findings were confirmed in humans across a broad range of age (18–70 years), body mass index (24–78 kg/m^2), and HBA1c (4.7%–14%) with diabetes and obesity producing a significant 71-fold ($P < 0.0002$) and 9-fold ($P < 0.0065$) reduction in the $CD26^{+}CD55^{+}$ subpopulation, respectively.

CONCLUSIONS: ASC subpopulation depletion is a novel mechanism for the variability seen in autologous therapies, correlates with the severity of comorbidities, and raises questions as to the suitability of autologous ASC therapies in patients with these comorbidities.

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Engrafted Stem Cells Promote Cascade Migration of Circulating Stem Cells via SDF-1a Pathway in Ischemic Tissue

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PURPOSE: During the past years, mesenchymal stem cells (MSCs) are reported to be recruited to the ischemic tissue and contribute to vascularization. Although the mechanism of migrated MSCs toward vascularization has been reported, the influence of these engrafted MSCs to the circulating stem cells is unclear. Here, we discuss the effect of engrafted MSCs in ischemic tissue toward the circulating MSCs.

METHODS: Expression of SDF-1a in hypoxic cultured MSCs was evaluated. MSCs from luciferase-Tg Lewis rats were transplanted intravenously into a rat lower limbhind ischemic model, whereas wild-type MSCs pretreated with/without SDF-1a shRNA were transplanted intramuscularly to ischemic tissue (MSC engrafted group/SDF-1aneg MSC engrafted group). The blood perfusion was evaluated with FLPI. The migration of circulating Luc-MSCs was tracked in vivo continuously by luminescence imaging. Hindlimb muscle sections were stained with anti-CD31, SDF-1a antibodies, and antiluciferase antibody to mark migrated MSCs.

RESULTS: SDF-1a expression was upregulated in MSCs under hypoxic culture and also found in engrafted MSCs in ischemic tissue. The bioimaging results showed that circulating MSCs migrated earlier in MSC engrafted group than control group. When blocking SDF-1a pathway in engrafed MSCs, the migration of circulating MSC was not significantly different as in control group. FLPI results showed that vascularization was significantly slowed when blocking SDF-1a pathway in engrafted MSCs. Immunohistologic staining showed that less migrated Luc-MSCs were found in ischemic tissue when engrafted MSC's SDF-1a pathway was blocked.

CONCLUSIONS: Engrafted MSCs produce SDF-1a in ischemic tissue promoting migrating cascade of circulating MSCs and accelerate vascularization.

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Hair Bulge Stem Cells Share a Tendon Stem Cell Lineage

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PURPOSE: Hair bulge stem cells contribute to wound healing, prompting interest in their identification and functional analysis. We show that hair bulge stem cells share a lineage with tendon stem cells, consistent with the proximity between the bulge and arrector pilli muscle.

METHODS: *Scx-cre/ROSAHIF2$^{fl/fl}$* mice were created to study bone morphogenetic protein signaling and hypoxia-inducible factor 2 in tendon development. Mice were incidentally noted to progressively lose dorsal hair, prompting evaluation of *Scx-cre* within skin and hair follicles using *Scx-cre/ROSA26^{mTmG}* lineage-tracing mice. Mechanism for hair loss was probed using immunostaining for phospho-SMAD1/5, a mediator of the hair cycle and bone morphogenetic protein signaling.

RESULTS: *Scx-cre/ROSAHIF2$^{fl/fl}$* mice develop their first coat normally followed by gradual loss apparent by 14 days after birth (A); *Scx-cre/BMPR1a$^{fl/fl}$* mice lose their hair within their first week. *Scx-cre/ROSA26^{mTmG}* lineage-tracing mice confirmed that the hair bulge was partially marked by cells of a tendon lineage (Scx-cre) (B and C). Within the skin, these cells were localized to the hair bulge. Histologic sectioning of skin from 4-week-old mice show increased pSMAD 1/5 expression within the bulge (D).

CONCLUSIONS: Our findings confirm that cells of a tendon lineage reside within the hair bulge and that these cells can regulate hair development and cycling. This unique model also suggests a previously undescribed relationship between hypoxia-inducible factor 2 and pSMAD1/5 signaling. Future studies will determine whether Scx-cre cells directly contribute to wound healing and hair regeneration.

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Adipose-Derived Stem Cells Seeded in a Soft Collagen Hydrogel Improve Healing in Murine Burns

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PURPOSE: Postburn scars and scar contractures have important functional and psychosocial implications for patients. Recently, stem cell therapy has gained attention as a potential therapeutic solution for this clinical challenge. Mesenchymal stem cells (MSCs) have been shown to stimulate angiogenesis, modulate inflammation, and improve wound healing. Our laboratory recently developed a soft collagen hydrogel that, when seeded with MSCs, improves MSC viability and augments their proregenerative capacity. By using a contact burn model in wild-type mice, we studied the effects of adipose-derived MSC (ASC)-seeded hydrogels on wound healing after thermal injury.

METHODS: Partial-thickness contact burns were created on the dorsum of wild-type mice. On days 5 and 10 after injury, burns were debrided and received either ASC hydrogel, ASC injection, hydrogel alone, or no treatment. On days 10 and 25, burns were harvested for histologic and molecular analysis.

RESULTS: ASC hydrogel-treated burns showed accelerated healing and time to reepithelialization, compared with ASC injection, hydrogel-alone or control burns. ASC hydrogel-treated burns exhibited increased vascularity, along with increased expression of the proangiogenic genes *MCP-1*, *VEGF*, and *SDF-1* at both the mRNA and protein level. Expression of the profibrotic gene *Timp1* and proinflammatory gene *Tnfa* were downregulated in ASC hydrogel-treated burns. Furthermore, ASC hydrogel-treated burns exhibited reduced scar area compared with hydrogel-treated and control wounds, with equivalent scar density.

CONCLUSIONS: ASC hydrogel therapy is effective for treating burns, with demonstrated proangiogenic, fibromodulatory, and immunomodulatory effects. Further refinement and testing is indicated to facilitate clinical translation.

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Release of Hand Contractures with the Percutaneous Aponeurotomy and Lipofilling Procedure: An Incisionless Regenerative Alternative to the FLAP

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PURPOSE: Needle pricks selectively cut tight structures while sparing looser neurovascular bundles. This allows a percutaneous mesh expansion of hand contractures, while subsequent seeding the mesh interspaces with fat grafts regenerates the gaps. We present our 10-year experience with percutaneous aponeurotomy and lipofilling (PALF), an alternative to flaps for hand contractures.

METHODS: With the contractures under tension, we percutaneously generate a pattern of 1.2-mm slits that mesh expand the contracture and then seed the generated scaffold with liposuctioned fat grafts. The hand is immobilized in extension for 5 to 7 days before returning to gentle activities.

RESULTS: We performed 246 PALF procedures (202 Dupuytren, 44 scar contractures) on 200 patients. No incisions or sutures were required. Patients had a quick recovery; 90% returned to gentle activities within 8 days. Dupuytren treatment yielded 110% and 57% correction at MPJ and PIPJ, respectively, at 12 months (comparable with open fasciectomy and flap). PALF-treated areas resulted in 30% tissue gain, allowing for incisionless release of contractures that would have otherwise required flap surgery. There was no nerve injury. Complications were infrequent and minimal.

CONCLUSIONS: One-mm needle pricks leave no scars; the sum of staggered slits can expand the overall meshed area by 20% to 30%. Fat grafting the tiny interspaces fills the gap with near-normal tissue, thus regenerating the tissue deficiency without scar or donor defect. Our experience shows that PALF is an incisionless, regenerative alternative to flaps.

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Macrophages from Visceral Adipose Tissue Contribute To the Onset of Diabetes

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PURPOSE: Obesity is characterized by increased visceral adipose tissue (VAT) and is the primary risk factor for type 2 diabetes. Both obesity and diabetes are now recognized as chronic proinflammatory diseases with the accumulation of macrophages. However, the cellular and molecular mechanisms that underlie the increase in VAT macrophages and contribution of these macrophages to diabetes remain unclear.

METHODS: We used fluorescent-assisted cell sorting to isolate $CD45^+CD11b^+F480^+$ macrophages from the VAT of normal, obese, and high-fat diet (HFD)-induced diabetic mice. $CD45^+CD11b^+CD66b^-$ macrophages from human subcutaneous adipose tissue and VAT from normal, obese, and diabetic patients were similarly isolated. Single-cell transcriptional analysis and RNA sequencing were used to determine polarization within the macrophages without previous surface marker bias. To identify the contribution of macrophages to diabetes, VAT macrophages were isolated from diabetic and normal mice and injected into the VAT of normal mice. Finally, to test for reversal of diabetes, VAT was surgically resected from diabetic mice, and the mice were placed on HFD or regular chow.

RESULTS: We observe an increase in bone marrow-derived proinflammatory VAT macrophages releasing interleukin-6, interleukin-1b, and tumor necrosis factor with the onset of diabetes. Normal mice injected with these proinflammatory macrophages in the presence of HFD develop diabetes significantly faster. Resection of VAT from HFD-induced diabetic mice in the presence of normal chow significantly quickens reversal of diabetes.

CONCLUSIONS: Our results suggest that proinflammatory bone marrow-derived macrophages in the VAT contribute to diabetes. Directed intervention of these cells will have implications in treating diabetes.

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Impact of Methylglyoxal on Diabetes Induced Progenitor Cell Dysfunction

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PURPOSE: Reactive oxygen species generation represents a likely mechanism by which diabetes impairs stem- and progenitor-cell function. Here, we determine the specific contribution of methylglyoxal, a highly potent reactive oxygen species that increases in the presence of hyperglycemia, on progenitor cell dysfunction and impaired wound healing in diabetes.

METHODS: We address methylglyoxal's impact within transgenic murine models of glyoxalase1 (Glo1) knockdown and overexpression, effectively manipulating methylglyoxal breakdown in vivo. Fluorescent cell sorting is employed to isolate progenitor cell populations from adipose tissue and bone marrow, which are further assayed utilizing single-cell microfluidic multiplexed reverse transcription polymerase chain reaction. Unsupervised hierarchical clustering is used to highlight and quantify dysfunctional subpopulations of progenitor cells.

RESULTS: Glo1 knockdown mice demonstrate depletion of $CD45^{-}CD31^{-}CD34^{+}$ ASC populations (2.74%) when compared with wild-type control mice (3.25%). However, they show greater number of adipose-derived stem cells compared with diabetic mice (1.14%). Similarly, Glo1 knockdown mice demonstrate fewer numbers of Lin-Sca+ckit+ hematopoietic stem cells (0.36%) when compared with wild-type mice (0.53%) and a greater number of these stem cells compared with diabetic mice (0.30%). We are currently testing both progenitor cell dysfunction in Glo1-depleted mice at a single-cell level and the reversal of such diabetic perturbations with Glo1 overexpression.

CONCLUSIONS: Although only partly representing the full oxidative load within a diabetic animal, methylglyoxal remains an extremely potent glycation agent capable of exerting significant negative impact on progenitor cells despite low concentrations. Our findings help address the utility of directed intervention in methylglyoxal catabolism, outlining avenues by which to explore new therapeutic targets for diabetic complications.

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BMPR-1A+ Adipose-Derived Stromal Cells Demonstrate Enhanced de novo Adipogenesis

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PURPOSE: Within the stromal vascular fraction of lipoaspirate resides a heterogeneous population of adipose-derived stromal cells with potential for de novo adipogenesis. Importantly, previous reports have demonstrated signaling through bone morphogenetic protein (BMP) receptor type 1A (BMPR-IA) to promote adipogenic lineage commitment among mesenchymal cells. In this study, we evaluated the ability for the BMPR-IA subpopulation of adipose-derived stromal cells to undergo enhanced formation of mature adipocytes.

METHODS: Freshly harvested human stromal cells were separated into BMPR-IA+ and BMPR-IA− fractions using magnetic-activated cell separation, and flow cytometry was performed to confirm enrichment. These cells, along with unsorted cells, were then cultured in adipogenic differentiation medium for 7 days followed by Oil-Red-O staining and gene expression analysis. Cells from each group were also labeled with a GFP lentivirus and transplanted into the inguinal fat pads of immunocompromised mice for evaluation of de novo adipocyte formation within a natural adipogenic niche.

RESULTS: Oil-Red-O staining showed significantly more lipid droplet formation in BMPR-IA+ cells ($*P < 0.05$), and this was paralleled by increased expression of FABP4 ($*P < 0.05$), a marker of terminal adipogenic differentiation, among BMPR-IA+ cells. Confocal microscopy of explanted inguinal fat pads demonstrated significantly more mature GFP+ adipocytes formed by BMPR-IA+ cells compared with BMPR-IA− and unsorted stromal cells ($**P < 0.01$).

CONCLUSIONS: BMPR-IA+ stromal cells show greater adipogenic ability both in vitro and in vivo. Enrichment for BMPR-IA+ cells may thus identify a subpopulation with increased utility in soft tissue restorative procedures where inclusion of proadipogenic stromal cells (eg, cell-assisted lipotransfer) may enhance reconstructive outcomes.

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Identification and Isolation of Proangiogenic Stromal Cells for Targeted Improvement of Fat Graft Retention

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PURPOSE: Enrichment of lipoaspirate with stromal cells may enhance fat graft retention, but the functional heterogeneity of these supplemental cells complicates this approach. Studies, however, have suggested a paracrine role for stromal cells within fat grafts promoting revascularization, and identification of a subpopulation with enhanced angiogenic potential may yield an optimal subset for use in soft tissue reconstructive strategies.

METHODS: Single-cell transcriptional profiling for angiogenic and cell surface marker genes was performed on human fat-derived stromal cells. Soft partitioning was performed to define an angiogenic cluster of interest and linear discriminant analysis identified correlating surface markers. Cells were then isolated through flow cytometry based on CD248, the strongest correlating marker. Gene transcriptional analysis was performed on $CD248^+$, $CD248^-$, and unsorted cells to confirm angiogenic gene expression patterns. Finally, the proangiogenic capacity of $CD248^+$ cells was tested using an endothelial tube formation assay and a mouse model of cell-assisted lipotransfer.

RESULTS: Flow cytometry revealed $CD248^+$ cells to comprise 16% of the overall heterogeneous stromal cell population. quantitative real-time polymerase chain reaction revealed $CD248^+$ cells to express significantly higher levels of VEGFa and HGF compared with $CD248^-$ and unsorted cells. $CD248^+$ cells also promoted increased endothelial tube formation and improved fat graft retention in vivo.

CONCLUSIONS: Single-cell transcriptional profiling identified multiple markers associated with stromal cell angiogenic capacity, with CD248 being the strongest. $CD248^+$ cells were found to have significantly increased angiogenic potential, making these cells potentially useful to augment fat grafting strategies for soft tissue reconstruction and for other targeted cell-based therapies in hypoxic environments.

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The Effect of Motor Denervation on Skeletal Muscle Stem Cells

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PURPOSE: Resident stem cells in skeletal muscles (MuSCs) express the transcription factor paired box protein 7 (Pax7) and repair injured muscle. Previous studies have reported MuSC depletion after long-term denervation, possibly explaining the irreversibility of denervation atrophy, but did not quantify Pax7+ cells and did not perform functional studies. It remains unknown to what extent MuSC depletion limits recovery after delayed reinnervation. Determining whether MuSCs in denervated muscle survive and retain regenerative ability would direct future approaches to muscle regeneration in denervation injuries.

METHODS: A 4-mm segment of the left sciatic nerve in 3 C57Bl6 mice was removed. After 3 months, the bilateral lower leg muscles were harvested and weighed, and flow cytometry was used to deplete Sca-1/CD31/CD45 and select calcein/VCAM/ITGA7 cells. Pax7 staining confirmed MuSC identity.

RESULTS: Tibialis anterior muscles weighed 11.9 ± 0.6 mg after denervation compared with 49.5 ± 2.5 mg ($P < 0.0001$). Two distinct populations of MuSCs expressing either high levels of VCAM (VCAMhigh) or ITGA7 (ITGA7high) were observed. ITGA7high cells were relatively larger than VCAMhigh cells. More than 90% of MuSCs isolated from muscles expressed Pax7. Denervated MuSCs expressed higher levels of VCAM. MuSCs as a percentage of total sorted cells was $37.7\% \pm 7.0\%$ in denervated legs versus $30.8\% \pm 3.7\%$ in controls, $P < 0.23$.

CONCLUSIONS: Muscle denervation alters MuSC phenotype from quiescence toward activation. Resident MuSCs increase in number 3 months after denervation and may retain intrinsic regenerative capacity.