CONCLUSIONS: The application of Leupeptin post-hind limb ischemia led to greater preservation of hind limb muscles. We postulate that by inhibiting calpain, Leupeptin inhibits the pathways that trigger cell death leading to greater tissue preservation. Studies focusing on the gross and histologic changes in the arteries, veins, and nerves between these groups are currently being performed.

REFERENCES:
1. Kauvar DS, Baer DG, Walters TJ. Influence of systemic hypotension on skeletal muscle ischemia-reperfusion injury after 4-hour tourniquet application. J Surg Educ. 2007;64:273–277.
2. Wang GW, Yang H, Wu WF, et al. Design and optimization of a biodegradable porous vein conduit using microtubes as a guide for rat sciatic nerve defect repair. Biomaterials. 2017;131:145–159.

Transdermal Drug Delivery System for Deferoxamine Improves Vascularity and Fat Graft Take Postirradiation

Presenter: Mimi R. Borrelli, MD

Co-Authors: Ronak A. Patel, BS; Dre Irizarry, MD; Jan Sokol; Dung H. Nguyen, MD, PharmD; Arash Momeni, MD; Michael T. Longaker, MD, MBA, FACS; Derrick C. Wan, MD; Abra H.-T. Shen, NA

Affiliation: Stanford University, Stanford, CA

PURPOSE: Radiotherapy is an important curative and preventative oncologic treatment but causes significant collateral damage to healthy soft tissue in the radiation field. The long-term outcome of radiotherapy is pathologic tissue fibrosis, which distorts tissue esthetic appearance, impairs function, and can profoundly effect patient quality of life. Fat grafting is gaining popularity as a technique able to restore and regenerate irradiated tissue. Grafted fat, however, is often poorly retained in the damaged and hypovascular recipient site. We have previously shown that preconditioning the irradiated tissue with subcutaneous injections of deferoxamine (DFO) can improve perfusion and subsequent fat graft retention. Repeated subcutaneous injections, however, may be painful and further irritate the recipient site. In this study, we therefore explored whether DFO preconditioning using a transdermal drug delivery system (TDDS) could reverse radiation-induced soft tissue damage and enhance subsequent fat graft survival.

METHODS: Female CD-1 nude mice underwent external beam irradiation of the scalp with 30 Gy fractionated in 5 Gy doses every 2 days for a total of 12 days. After a 5-week recovery period, mice either received a DFO–TDDS or a control carrier TDDS, which was replaced changed every other day for a 2-week period. Laser Doppler analysis was recorded before irradiation, following irradiation, and 24 hours following each TDDS treatment. Human liposapirate was then injected into the subcutaneous plane of the scalp (200 µl/graft), and fat graft retention was monitored radiographically every 2 weeks for 8 weeks total, at which point the skin and transplanted fat were harvested for mechanical strength testing and histologic analysis. Blood samples were obtained from mice receiving the DFO–TDDS after 24 hours to assess systemic levels of DFO by mass spectrometry.

RESULTS: The DFO–TDDS application resulted in significantly increased perfusion at the recipient site, as indicated by both laser Doppler analysis and CD31 immunofluorescent staining. The mice receiving DFO treated also had reduced skin stiffness, and significantly greater fat retention compared to the mice receiving the control carrier TDDS. Intravenous systemic levels of DFO were below a quantifiable level at 24 hours after DFO–TDDS placement.

CONCLUSIONS: Transdermal DFO delivery offers an effective and noninvasive mechanism to improve perfusion and reduce stiffness of irradiated tissue. These findings are also associated with improved fat graft retention at irradiated, DFO-preconditioned recipient sites making this approach promising in the reconstruction of postoncologic irradiated soft tissue defects.

Model of Radiation-induced Hind Limb Contracture and Skin Fibrosis Rescued by Fat Grafting

Presenter: Mimi R. Borrelli, MD

Co-Authors: Ronak A. Patel, BS; Jan Sokol; Dre Irizarry, MD; Abra H. Shen, SB; Dung H. Nguyen, MD, PharmD; Arash Momeni, MD; Michael T. Longaker, MD, MBA, FACS; Derrick C. Wan, MD
**Affiliation:** Stanford University, Stanford, Calif.

**INTRODUCTION:** Radiotherapy is an effective anti-cancer treatment, able to reduce tumor size and decrease local cancer recurrence. However, the long-term outcome of radiotherapy is significant and pathologic fibrosis of the soft tissue surrounding the malignancy. Radiation-induced soft tissue fibrosis can disrupt tissue esthetic appears and impair function, such as impaired swallowing and limb contracture. Fat grafting is gaining popularity as a surgical technique able to prevent or reverse the radiation-induced soft tissue fibrosis. We developed a mouse model of radiation-induced hind limb contracture and investigated the potential of grafted fat to restore mobility to the irradiated hind limb.

**METHODS:** The hind limbs of Prrx1Cre;R26mTmG mice were irradiated with 30 Gy fractionated in 5 Gy doses every 2 days for a total of 12 days. The Prrx1Cre;R26mTmG mice were used to label a fibrogenic subpopulation of fibroblasts in ventral skin (PRRX-1+) by embryonic expression Cre. A 4-week period followed irradiation to allow limb contracture to develop, and mice were then sacrificed, and hind limbs were processed for histology. To explore the therapeutic effects of fat grafting, CD-1 nude mice were irradiated with the same irradiation protocol, and at 4 weeks, the mice were injected with 200 μl of human lipoaspirate fat or lipoaspirate enriched with stromal vascular cells (10,000 cells/200 μl) directly into the subcutaneous space on the ventral surface of the irradiated hind limbs. We used 2 control mice; mice injected with 200 μl of saline or mice who received sham surgery with no injection. Limb extension was measured every 2 weeks for a total of 12 weeks, and mice were then sacrificed for hind limb skin mechanical strength testing and histologic analysis.

**RESULTS:** Hind limb irradiation significantly reduced limb extensibility compared to the nonirradiated side, and contracture was associated with a significant increase in the fibrogenic Prrx1+ fibroblast subpopulation in mouse ventral skin. Fat grafting progressively increased limb extension, reduced skin stiffness, and reversed the fibrotic histologic changes in the skin. The greatest improvements were found in mice who received fat grafted with stromal vascular cells.

**CONCLUSION:** We present a mouse model of radiation-induced hind limb contracture which we use to show that grafted fat can reverse the fibrotic changes seen in irradiated skin and can improve the extensibility of contracted limbs postirradiation.

**Novel Xenografting Model to Explore the Mechanisms Mediating Acute and Chronic Fibrosis in Human Skin Fibroblasts**

**Presenter:** Mimi R. Borrelli, MD

**Co-Authors:** Abra H.-T. Shen; Shamik Mascharak, BS; Heather E. desJardins-Park, AB; Ronak A. Patel, BS; Jan Sokol; Derrick C. Wan, MD; Michael T. Longaker, MD, MBA, FACS; H. Peter Lorenz, MD

**Affiliation:** Stanford University, Stanford, CA

**INTRODUCTION:** Human scar formation and fibrosis are difficult to accurately recapitulate using mouse models, given the significant anatomical and physiologic differences between mouse and human skin. Xenografts of human skin on immunodeficient mice provide an accessible means of assessing human skin’s physiology and response to wounding or fibrosis-inducing conditions in vivo. However, current xenograft models are limited by poor engraftment rates and inability to specifically explore the mechanisms mediating fibrosis in human fibroblasts.

**OBJECTIVE:** We describe a novel skin xenografting model to investigate the response of human dermal fibroblasts to different fibrosis-promoting conditions.

**METHODS:** Full-thickness circular 8-mm human foreskin samples were sutured into the dorsum of P2 immunocompromised (NSG) mice as subcutaneous grafts (n = 30), and surgically exposed after 7 days to produce cutaneous grafts. Successful engraftment and preservation of normal skin physiology were confirmed by histology. Machine learning–based assessment of collagen fiber networks from stained skin histology specimens was achieved using a novel computational algorithm developed by our laboratory. To study the acute fibrotic response, 4-mm partial-thickness wounds were created within the xenografts using a biopsy punch; wounds were monitored until closure. To explore the chronic fibrotic response, xenografted skin was irradiated with 30 Gy fractionated into six 5 Gy doses delivered every other day for a total of 12 days. Following radiation, chronic fibrotic changes were allowed to develop over an interval of 4 weeks. At the respective endpoints, xenografted skin was harvested for histology. Human fibroblasts were isolated using flow cytometry with a negative gating strategy to exclude mouse and human hematopoietic cells (CD45/Ter-119/CD235a), endothelial cells (CD31⁺), and epithelial cells (CD326 [mEpCam]/mCD324 [E-Cadherin]), and a positive gate to include only human fibroblasts (CD90⁺). Microarray analyses were used to compare gene expression of human fibroblasts isolated from scarred/irradiated xenografts to those from unwounded/non-irradiated xenografts.