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**INTRODUCTION:** Radiotherapy is an effective anticancer 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 Prx1Cre;R26mTmG mice were irradiated with 30 Gy fractionated in 5 Gy doses every 2 days for a total of 12 days. The Prx1Cre;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 Prx1+ 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

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**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/CD235α), 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/nonirradiated xenografts.
RESULTS: Xenografted foreskin was structurally similar to native (ungrafted) foreskin on histology. Collagen fiber network analysis confirmed that xenografted skin was more similar to foreskin than to scarred adult human skin. Wounds created in xenografted skin exhibited slower wound closure compared to stented mouse wounds, indicating healing primarily via formation of granulation tissue (akin to human skin) rather than contraction (typical of mouse skin). Irradiated skin was significantly indurated on histologic assessment, consistent with chronic irradiation damage/fibrosis. Immunofluorescence staining confirmed successful xenograft vascularization and survival of human skin cells and human origin of granulation tissue. Gene expression analysis of fibroblasts isolated from acutely and chronically fibrosed xenografts revealed upregulation of the Wnt and FAK pathways and increased expression of the CD26 surface antigen.

CONCLUSIONS: We present a novel foreskin xenografting model and demonstrate its utility in specifically investigating the in vivo human fibroblast response in acute and chronic fibrosis. This model provides an accessible and informative tool to aid in elucidation of fibroblast-driven mechanisms responsible for scarring and fibrosis. Ultimately, this work may enable the discovery of novel cellular and molecular targets to reduce skin scarring and fibrosis.

Understanding the Mechanism of In Vitro Chondrogenesis Using a Coculture System for Generating a Tissue-engineered Cartilage Construct

Presenter: Mary E. Ziegler, PhD

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INTRODUCTION: Microtia is a congenital condition that results in external ear deformities of varying degrees, of which the most extreme form is anotia. The current surgical reconstruction techniques all require a multi-stage approach and have other intrinsic disadvantages. Cartilage engineering is an alternative treatment approach in the field of external ear reconstruction. Typically, this method uses chondrocytes isolated from the remnants of auricular cartilage from the patient, which are cultured in the laboratory with the aim of creating bioengineered cartilage matrices. However, cartilage engineering is plagued with a variety of challenges, including the difficulty in culturing sufficient chondrocytes and the production of cartilage that does not possess the proper mechanical properties. To overcome these difficulties, we created a novel cartilage engineering model that involves coculturing chondrocytes with adipose-derived stem cells (ADSCs). These cells are seeded onto a 3-dimensional (3D) allograft adipose-derived extracellular matrix scaffold (AAM) at a defined ratio, stimulating chondrogenesis. However, the mechanism of how this happens is unclear. We hypothesized that the ADSCs have a trophic effect on the chondrocytes, making them more chondrogenic compared to the culturing of chondrocytes alone.

MATERIALS AND METHODS: Auricular chondrocytes (ACs) were isolated from porcine ear, and ADSCs were isolated from human lipoaspirate. ACs and ADSCs were cocultured either directly or via a transwell system at the defined ratio. In addition, the cells were cultured alone. The supernatants from these cultures were collected, and a secretome analysis was performed to assess the differential secretion of proteins under these conditions. The experiment was also conducted in the presence of AAM.

RESULTS: The secretome analysis revealed that the cells in the coculture conditions produced more chondrogenic factors compared to the chondrocytes alone. In addition, we validated that the factors important for chondroinduction were being secreted by the ADSCs.

CONCLUSION: These data revealed that the ADSCs provided paracrine support to induce chondrogenesis, which supports cartilage engineering when the number of ACs available is limited. Furthermore, uncovering the mechanism of chondrogenesis in this setting provides clues for improving cartilage engineering to ultimately utilize a patient’s own chondrocytes and adipose-derived cells for the creation of a customized ear framework that could be used for further surgical reconstruction.

Differential Gene Expression in Nerves Repaired Under Low and High Tension

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PURPOSE: Tension has been shown to be detrimental to nerve regrowth and reinnervation after primary nerve repair, likely via local ischemia and aberrant wound healing. We hypothesize that an improved understanding of the mechanisms underlying pathologic nerve healing may provide insight into potential targets for optimizing outcomes after nerve repair. The purpose of this study was to use...