We have developed a low-cost tissue-engineering perfusion circuit that facilitates 3D-tissue-culture while allowing for repeated live-imaging as the cultured tissue develops. The instructions for our setup can be utilized to replicate our devices in other labs, and these designs can be readily customized to meet the needs of specific experimental aims.

QS3

CRISPR/Cas9 Editing of Autologous Dendritic Cells to Enhance Angiogenesis and Wound Healing

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Purpose: Dendritic cells (DCs) are a heterogeneous cell population which critically regulates the adaptive immune response. Depending on their activation status, DCs can also promote peripheral immune tolerance, thus limiting the activation of the immune system and tissue damage. The N-myc downregulated gene 2 (Ndr2) is highly expressed in DCs and limits the secretion of vascular endothelial growth factor (VEGF), which is critical for wound healing. Cell-based therapy approaches using DCs have been approved by the FDA and clinical trials using DC immunotherapy are being performed against a variety of cancer types. However, the role of DC therapy for wound healing has not yet been investigated.

Methods: Hematopoietic progenitor cells were isolated from the bone marrow of mice and differentiated into DCs over a 7-day in vitro culture period. Pharmacologic down-regulation of Ndr2 was performed by treatment of cultures with 1,25-dihydroxyvitamin D3 (VD3) and the angiogenic potential of the treated cells was evaluated by endothelial cell (EC) tube formation assays. Cytokine secretion of DCs was measured in the conditioned media using Luminex multiplex assays. To permanently knock out Ndr2 in DCs, a CRISPR/Cas9 gene editing approach was developed, using Cas9/sgRNA-ribonucleoproteins and electroporation. The determine the impact of genetically edited DCs on wound healing, splinted excisional wounds in C57BL6/J (wild-type) mice were treated weekly with pullulan-collagen hydrogels seeded with Ndr2-knockout (KO) DCs, control DCs which had undergone electroporation only, or blank hydrogels. The transcriptomic impact of Ndr2 downregulation on DC fate was evaluated by microfluidic single-cell RNA sequencing (scRNA seq) of Ndr2-KO DCs, VD3-treated DCs and control DCs.

Results: Ndr2 down-regulation lead to a significantly stronger EC tube formation in co-cultures with VD3-treated DCs, and strongly enhanced VEGF secretion compared to untreated DCs in vitro. A CRISPR/Cas9 editing pipeline was developed for KO of Ndr2 in DCs with a transfection rate and editing efficiency of > 90% shown by Sanger Sequencing. Excisional wounds treated with Ndr2-KO DCs demonstrated significantly accelerated healing compared to control DCs and blank hydrogels. scRNA seq revealed that Ndr2 downregulation strongly induced VEGF expression and anti-oxidant transcriptomic signatures.

Conclusion: Our data indicate that KO of Ndr2 in DCs strongly enhances their secretion of VEGF, thus promoting angiogenesis and accelerating wound healing. Given the ready availability of DCs from the human blood through established leukapheresis protocols and easy multiplication in vitro, CRISPR/Cas9 editing of DCs is a promising new approach to induce wound healing and soft-tissue regeneration.

QS4

In Vivo Quantitative Analysis of Subcutaneous Membranous Layers -Superficial And Deep Fascia- In Eleven Regions of the Human Body

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**Purpose:** Surgical Site Infections (SSIs) and Hypertrophic scars (HSs) are the most common complications of wound healing. Most SSIs are superficial infections involving the skin and subcutaneous tissue (SQ) only. Abnormal scaring is driven by ongoing dermal inflammation in high skin tension areas (e.g. anterior and posterior chest). For prevention, proper suturing techniques are required, in particular for subcutaneous adipose tissue, to reduce high skin stretching tension and prevent ischemia. While adipose lobules cannot be sutured, superficial fascia (SF) -the membranous structure of adipose stroma- must be sutured. However, the exact anatomy and characteristics such as number and thickness of SF throughout the body regions are still lacking. This is the first study to present a detailed quantitative analysis of SF anatomy. We believe such details will help in optimization of subcutaneous sutures.

**Methods:** Superficial and deep fascia (DF) were analyzed using ultrasound imaging in predefined 73-point locations, distributed among eleven body regions of ten healthy male volunteers; Anterior chest: 9 points, abdomen: 10, posterior chest: 9, lumbar region: 6, gluteus: 2, arm: 8, elbow: 3, forearm: 8, thigh: 9, knee: 3 and leg: 6. Using ImageJ software, thickness of SF and DF layers, dermis and SQ were measured along SF percentage. Three random measurements were taken for each variable then averaged and used this average for statistical analysis. In addition, number of SF layers was counted, total thickness of SF was calculated by summing the average thickness of all SF layers and total membranous layers thickness by summing total SF and DF thicknesses.

**Results:** Overall, 730 means were analyzed with multilevel mixed linear model for all variables except average layer thickness of SF which had 1635 means; since each point had one or more layers of SF, DF and dermis were significantly thickest in posterior chest region which had the highest layer thickness of SF measuring 0.64 ± 0.01 mm. Anterior chest and gluteus had the highest content of SF due to having the highest layers number (3.67 ± 0.08, 3.45 ± 0.143), yet significantly thickest gluteus SQ and lowest SF percentage. SF changed inconsistently within subcutaneous adipose tissue; SF, DF and dermis jointly handles stretching tension, therefore, to understand the effect of environment, analysis of the variable’s interaction was performed and showed significant accelerated increase in the thickness of SF and dermis in anterior and posterior chest as compared to lower tension regions (all p<0.001).

**Conclusion:** Our results showed that dermis and subcutaneous membranous layers tend to be thick in the high-tension areas such as the upper trunk. It was suggested that SSIs and HSs could be prevented by realizing the tension applied on the operated area; finding then suturing the membranous layers during the operation.

**QS5**

**Cryopreserved Adipose for Hypodermal Augmentation After Full-thickness Burns**

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**Purpose:** Burn and blast injuries to the face and extremities are highly morbid injuries affecting quality of life, ability to work, and psychosocial well-being. Without exception, extensive burn injuries require surgical debridement, with standard of care reconstruction involving autologous skin grafting to restore cutaneous integrity. This treatment modality is limited in extensive burns or in highly visible areas by lack of donor site and/or soft tissue deficits resulting in significant disfigurement. Hypodermal restoration via autologous adipose transplantation provides padding for the overlying skin, helps restore native features, and enhances contour and texture. However, this technique is limited by graft retention and often requires multiple rounds of grafting and consequently, multiple rounds of surgery, each with separate anesthesia, to achieve adequate results. The goal of this study was to demonstrate the therapeutic validity and efficacy of utilizing cryopreserved adipose to avoid multiple liposuction events when serial skin and fat grafting procedures are performed to restore epidermal, dermal, and hypodermal integrity after full-thickness burn.

**Methods:** Adipose was collected from female Yorkshire swine and processed day-of-collection for immediate cryopreservation. This adipose was preserved for 3 months prior to initiation of the next stage of the experiment. After three-month lapse, female Yorkshire swine received 16, 4x4 cm full-thickness burns using an electric brand. After 48 hours, eschar was removed down to fascia. Skin grafts were collected as split-thickness skin grafts. The pigs were maintained for 8 weeks from time of engraftment and interval serum, photography, ultrasound, and biopsies were collected. At 8 weeks post-engraftment animals were sacrificed