PURPOSE: Lymphedema affects over 140 million individuals worldwide and can be caused by surgical injury or resection of lymph nodes. Patients with lymphedema are at risk for infection, impaired extremity function, and in rare cases malignant transformation. Pathogenesis begins with dysfunctional lymphatic drainage which then leads to fluid stasis, inflammation, fibrosis, and progressive stiffness of the soft tissue. We hypothesize that a progressive increase in interstitial pressure has an adverse effect on lymphatic endothelial cell function, thus further contributing to disease progression. To test this hypothesis, we cultured lymphatic endothelial cells (LEC) in 3-D scaffolds of different stiffness conditions to determine the effect of interstitial pressure on LEC proliferation and tube formation.

METHODS: Human primary LECs were isolated from neonatal foreskin under an IRB approved protocol and cultured in Endothelial Cell Basal Medium (EBM, Lonza) with 15% FBS. LECs (1x10⁴) were encapsulated in a range of percentages of biomimetic collagen hydrogels with physiologically relevant pressure including: 5.25% (3.37 pKa), 6.0% (5.57 pKa), 7.5% (12.8 pKa), and 9.0% (15.31 pKa). Experiments were performed in quadruplicate. 3-D in vitro cultures were observed for their phenotypic behavior and analyzed at defined time points for three weeks. Two-way ANOVA (Tukey’s multiple comparison test) was used for multi-group statistical analysis.

RESULTS: Lymphangiogenesis, lymphatic vessel-like branching, and formation of collateral lymphatic-like structures were observed at 3 days post cell-seeding in 3D hydrogel. Immunofluorescence demonstrated that these branch-like structures were positively for podoplanin an LECs specific marker. The optimal percentage of gel concentration which favored the formation of these structures was 6.0% gel. Using group comparisons, 6.0% and 7.5% groups were statistically different from 5.25% and 9.0% groups (p = 0.0198). There was no statistical difference between 6.0% and 7.5% groups, and 5.25% and 9.0% groups.

CONCLUSION: The physiologic response to lymphatic injury and lymph fluid obstruction involves lymphangiogenesis and formation of collateral vessels to provide alternative drainage pathways. As lymphedema progresses towards a fibrotic stage, interstitial pressure increases. In this study, we used a 3D in vitro model to demonstrate that lymphangiogenesis occurs optimally at 6.0–7.5% gel (5.57 to 12.8 kPa), which is in the normal physiologic range. At lower (3.37 kPa) or higher (15.31kPa) pressures, lymphangiogenesis is less efficient. This knowledge may be used to guide medical/pro-lymphangiogenic and surgical approaches to the management of lymphedema.

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Functional Characterization of Fibroblasts Differentiated from Adipose-Derived Stem Cells

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PURPOSE: Several dermal wound healing applications have been used to test the therapeutic potential of Adipose-derived stem cells (ASCs); however, we have yet to see dramatic improvement in healing time or scar preventing in a large scale clinical trial. Here, we characterize the fibroblastic differentiation capacity of these cells and extracellular matrix (ECM) production compared to primary cutaneous fibroblasts as recent literature suggests the wound healing capabilities and ECM production of fibroblasts differentiated from ASCs (dFib cells) may be superior.

METHODS: ASCs and primary fibroblasts were isolated from healthy female patients undergoing abdominoplasty (n=8, 45.14±14.16 years old). ASCs underwent fibroblastic differentiation via incubation with Connective Tissue Growth Factor and Ascorbic Acid for 3 weeks. Fibroblasts and dFib cells were then allowed to reach confluence and produce ECM. Proliferating fibroblasts and dFib cells were assayed for ASC and fibroblast markers to confirm differentiation. Further, mRNA and protein were harvested on Days 1, 7 and/or 21 of ECM production to quantify healthy and scar ECM marker expression via qPCR. Additionally, cells underwent in vitro scratch test migration analysis and subsequent Masson’s trichrome stain to evaluate migration and defect closure.

RESULTS: Differentiated cells showed increased RNA expression of the fibroblast marker Ephb3 (6.94±1.98-Fold, p<0.05) and decreased expression in the stem cell markers CD34 (0.35±0.23-Fold, p<0.01) and CD105 (0.65±0.18-Fold, p<0.01) when normalized to ASC
expression which parallels primary fibroblast expression levels (4.61 ± 1.8-Fold, 0.027 ± 0.03-Fold, and 0.22 ± 0.17-Fold respectively, p<0.05). dFib cells also showed increased RNA expression of healthy ECM marker genes Fibronectin (0.93 ± 0.28-Fold, p<0.05) and Collagen 1 (4.67 ± 1.4-Fold, p<0.05), and Elastin (0.93 ± 0.63-Fold, p<0.01) compared to primary fibroblasts (0.62 ± 0.16-Fold, 2.21 ± 0.86-Fold, and vs. 0.27 ± 0.08-Fold respectively). Proliferating dFib cells further showed differential expression compared to fibroblasts for the scar tissue markers αSMA (0.011 ± 0.006-Fold vs. 0.024 ± 0.012-Fold, p<0.05), Collagen III (0.72 ± 0.2-Fold vs. 0.26 ± 0.11-Fold, p<0.001), and TIMP-1 (2.33 ± 0.63-Fold vs. 0.6 ± 0.18-Fold, p<0.001). Scratch test assays revealed dFib cells maintain smaller defects throughout the healing time course with more cells migrating into the defect. Finally, dFib cells closed the defects significantly faster than primary cutaneous fibroblasts (32 ± 12.85 hours vs. 64 ± 13.85 hours, p<0.01). Similarly, Masson’s Trichrome staining demonstrates smaller defects after 3 weeks of recovery using dFib cells compared to primary fibroblast (1.04 ± 0.13mm² vs. 1.29 ± 0.39 mm²) however, this difference did not reach significance (p=0.16).

CONCLUSION: ASCs can be differentiated into fibroblast-like cells. These cells produce a robust ECM more similar to healthy skin as opposed to the scar tissue produced by primary cutaneous fibroblasts. These cells migrate into and close in vitro scratch defects more quickly than primary cutaneous fibroblasts and trend toward smaller long-term wounds. ASC differentiated fibroblasts show initial promise for regenerative medicine applications and should be investigated further for optimization in cutaneous wound healing and other therapeutic applications.

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Multi-Scale Modeling of Tissue Expansion: Genome Expression Patterns in the Acute Stretch Scenario

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PURPOSE: Despite decades of experience, tissue expansion (TE) often has high complication rates. Creating a reliable model of skin growth would allow for data-driven optimization of expansion protocols and decrease complication rates if used to plan the expansion. The changes in signaling pathways observed on the transcriptional level in skin under stretch are not well understood. Therefore, we combined mathematical models of skin under stretch with the biological response measured by gene expression levels and with histological assessment of skin structure with the goal of creating a comprehensive multi-scale model of tissue expansion.

METHODS: Five animal models (Yucatan minipigs) underwent 10 expansion protocols. Each animal was tattooed with 4 grids, 2 of which served as controls. Expanders were placed subcutaneously. The expansion protocols varied regarding volume of fill (60 or 30 cc), timing (1 hour, 24 hours, 3 days, or 7 days prior to expansion), or single versus 2 fills. 3D photography was captured for isogeometric analysis to measure skin growth and stretch. Total RNA from individual biopsies was isolated, gene expression was estimated using RNA-Seq (64 samples), then differences in gene expression were calculated and verified by qRT-PCR.

RESULTS: Statistically significant changes in gene expression levels correlated to the amount of stretch were obtained for each model. Illustrates the amount of stretch and growth attained prior to sacrifice, as measured by isogeometric analysis for model #3. The apex of the expander (orange) represents the highest stretch and was correlated with the largest changes in gene expression. The genes most dramatically activated by stretch include MMP1, SAA3, ILB1. PDLIM and RHOF were two of the most consistently down-regulated genes. The identified genes include well-known responders to the mechanical force (e.g. MMP1 or TNC), as well as completely new genes with no described role in skin adaption to stretch, presenting a new area for further study.