walls, (0.5 Pa, 1.5 Pa, 2.5 Pa). Cell morphology, alignment and migration were analyzed.

RESULTS: The ATC provided consistently stable environmental and temperature control throughout extended cultures. Live imaging revealed dynamic endothelia with cells migrating throughout the vessel walls, both downstream (with) and upstream (against) the flow direction. Cell-cell junctions were contiguous, indicating a confluent, healthy endothelium with intact cytoskeletons. The cross-sectional area of the microvessel expanded and changed profile from the original square cross-section defined lithographically toward an elliptical cross-section with a larger dimension. The displacement of migrating endothelial cells correlated positively with the applied shear stress, with maximum displacement occurring at the highest shear 2.5 Pa (25 dyne/cm²). Similarly, the endothelial cells elongated and aligned in the direction of flow, with net alignment increasing with the magnitude of shear.

CONCLUSIONS: This breakthrough sets the stage for clinical translation of the pre-vascularized tissues, provides new insights into relationships between hemodynamic forces and cell morphology/dynamics, and advances studies of mechano-biology that seek to identify the molecular mechanisms by which endothelial cells sense shear stress.

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Optimizing Vascular Invasion into Hydrogel Scaffolds Using Bioactive Sphingolipids

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PURPOSE: The promise of an ideal “off-the-shelf” regenerative template has long been predicated upon the availability of an adequate inherent vasculature. Due to this, current dermal regenerative templates such as Integra™ are clinically limited in scope secondary to their dependence on excellent host wound bed vascularity. This reliance in turn restricts their applicability in any but the most optimal wound beds, all but prohibiting their use in wound beds with exposed bone, tendon, or hardware. Recently, sphingosine-1-phosphate (S1P), a bioactive sphingolipid stored in platelets and found in micromolar quantities in the plasma and nearly absent in the interstitium, has gained widespread attention for its effects on endothelial motility and vascular maturation. In fact, it is thought that a reversal of the physiologic gradient causing S1P to be present in the interstitium, such as in wound healing, is responsible for inducing reparative angiogenesis. It is further hypothesized that gradients of S1P are a major signaling component in activating homeostatically nascent angiogenic mechanisms by promoting endothelial sprouting, motility, and stability. Herein we build upon our novel scaffolds by incorporating a bioactive sphingolipid into a collagen matrix in order to select for and amplify endothelial invasion to create a rapidly integrated, vascularized, tissue engineered construct with potentially profound implications for application in poorly vascularized wound beds.

METHODS: Raft cultures were created by seeding 100 ul of 3 mg/ml type I collagen onto 8um pore rafts. GFP labeled HUVECs were topically seeded and cultured for 7 days. The media in contact with the cells was devoid of S1P and a gradient in the collagen established by filling the bottom well in 1μM S1P. Gradients of VEGF, FGF, and concentrations of fetal bovine serum were also tested in the absence of S1P. Rafts were cross-sectioned at 100 um using a vibratome and sections stained with toluidine blue. Sections were quantified for number of sprouts per square micron, average length of sprouts, number of cells per structure, and number of junctions. Confocal reflectance z-stacks were taken to confirm the presence of lumens within the sprouts.

RESULTS: Robust and complex invasion was seen as early as day 3 in cultures containing S1P. S1P rafts demonstrated multicellular, luminal, complex sprouts that anastomosed to adjacent sprouts as early as day 14. At seven days of culture, these constructs exhibited an average sprout density of 37±8 sprouts/mm² with an average depth of invasion of 129±58 um. Origin endoluminal diameters were on the order of 10–20 um. Rafts without S1P exhibited confluent superficial growth but no invasion into the collagen matrix regardless of VEGF, FGF, or serum gradient.

CONCLUSION: With current dermal replacement products currently limited by an inadequate induction of endothelial invasion in poorly vascularized wound beds, we have herein demonstrated the remarkable ability of sphingosine-1-phosphate, a bioactive sphingolipid, to induce robust and
complex endothelial invasion in a collagen template. Future work will focus on the incorporation of this sphingolipid into our novel hydrogel microsphere drug delivery platform towards a powerful new dermal regenerative template capable of inducing endothelial invasion.

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A Three-Dimensional Tissue Engineered Platform to Analyze the Effect of Various Microvascular Shear Stress Conditions

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PURPOSE: Open deep tissue wounds commonly result from severe burns, trauma, chronic diseases, irradiation, and infection. Autologous tissue transfer remains the gold standard for surgical reconstruction, but unfortunately can come with significant complications. The development of an on-demand, geometrically tunable tissue engineered substitute with an inherent vascular supply would transform reconstructive surgical practice, but regeneration of thicker or larger tissues of clinically relevant size remains a challenge due to poor oxygen diffusion into cells within these constructs. Hemodynamic shear stress alters cellular morphology and biological activity, especially luminal endothelial cells within blood vessels. However, these mechanisms have only been studied in isolation in vitro, and work within complex vascular networks has remained elusive. We have thus fabricated a unique three-dimensional vascular anatomy regime with various geometric angles to study the effects of differential shear stress on endothelial cell behavior.

METHODS: A multi-hairpin channel geometry with a diameter of 1.5 mm was designed in SolidWorks and created using a 3D-printer. Flow parameters were calculated using fluid simulations performed by Ansys Fluent to determine expected shear stresses throughout the channel. A Pluronic-F127 multi-hairpin channel was sacrificed in type-1 collagen creating a central microchannel with encapsulated human placental pericytes in the collagen bulk. Subsequently, human aortic smooth muscle cells were intraluminally seeded into the macrochannel, followed by human umbilical vein endothelial cells 24 hours later. Constructs were statically cultured for 72 hours followed by dynamic perfusion at 10 dynes/cm² for 10 days. After 14 days of culture, constructs were analyzed for changes in endothelial phenotype. Images were analyzed in ImageJ (NIH).

RESULTS: Fluid simulation verified the presence of different shear stresses at various regions in the channel. Acute angles provided regions of higher shear stress, and the plenum facilitated higher vorticity. The geometry allowed for multiple shear regimes within the construct for high throughput analysis. MicroCT analysis revealed the development of appropriately shaped microchannels that recapitulated the 3D printed geometry. Hematoxylin and Eosin staining revealed the adherence of vascular cells along the microchannel. Regions of interest were stained with DAPI and confocal microscopy was used to take scans of the whole section. Images were converted to binary and the distribution of bulk cells around the vascularized microchannel was determined. Distribution of bulk cells was found to primarily localize around the microchannel, with 39% of cells being within 500 μm of the microchannel. Although bulk cells were originally randomly mixed into a homogenous distribution, the localization of bulk cells suggested that the presence of shear stress promoted bulk cellular migration.

CONCLUSION: Using our novel platform, we have fabricated an anatomically appropriate vascularized model with different geometric angles to study flow dynamics and the effects of differential shear stress on recruitment of bulk cells to stabilize the neovessel. This physiological model overcomes the limitations of previous 2D and 3D flow studies with the potential to recapitulate in-vivo cell organization. We have integrated the capabilities of 3D printing and tissue engineering to develop printable biologically derived, cell-friendly hydrogels.

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Transcriptome Analysis of Fetal Versus Adult Hair Follicle Dermal Papilla Cells Reveals Key Differences to Explain the Lack of Human Adult Hair Follicle Regenerative Capacity

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