and will use our technique to create 3D organoids capable of transplantation.

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A Novel Technique for Tissue Engineering Periosteum Using Three-Dimensional Bioprinting

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BACKGROUND: Periosteum plays a vital role in bone repair by housing a population of cells with remarkable regenerative capacity, including osteoblasts and mesenchymal stem cells. Periosteal flaps are often used by reconstructive surgeons to promote healing of bone defects caused by trauma, congenital deformities, or tumor resection. However, the utility of periosteal flaps is limited by donor-site availability and morbidity. This study presents a unique method for periosteum tissue engineering in which a three-dimensional (3D) bioprinter was used to print a precise pattern of periosteal derived cells (PDCs) onto collagen scaffolds.

METHODS: PDCs were isolated from the periosteum of bovine femurs and expanded in culture. PDCs were then mixed with alginate gel to create a bio-ink, which was printed in three different experimental groups: bio-ink alone; bio-ink printed on a type I collagen (COL1) scaffold; and bio-ink printed on a type II collagen (COL2) scaffold. To maximize the viability of the PDCs, the bio-ink was printed in a precise lattice pattern that was created using computer-aided design (CAD) software. PDCs were also cultured in monolayer (no alginate, no collagen) to serve as a control group. All groups were incubated in culture media and evaluated at one and two week time points. Live/Dead staining was used to assess cell viability. Polymerase chain reaction (PCR) was used to quantify gene expression and assess osteogenic differentiation.

RESULTS: On gross examination, the COL1 and COL2 scaffold groups maintained greater structural integrity than the bio-ink only group. Live/Dead imaging showed high viability of cells at one and two weeks in all experimental groups. PCR results demonstrated an increase in gene expression of the osteogenic differentiation markers osteocalcin (OCN) and alkaline phosphate (ALP) in all treatment groups relative to the monolayer control group. OCN expression was most significant in the COL1 group. PCR also showed an increase in COL2 gene expression in all treatment groups, but most significantly in the COL2 scaffold group.

CONCLUSION: The results presented here support a novel method for using 3D bioprinting to engineer periosteum constructs. The COL1 and COL2 scaffolds promoted cell viability and structural stability. Increases in OCN and ALP gene expression suggest the PDCs were undergoing osteogenic differentiation, with the COL1 scaffold being most supportive of this phenotype. The increase in COL2 gene expression, a chondrogenic marker, suggest some of the PDCs may be undergoing early chondrogenic differentiation before endochondral ossification into bone. In vivo studies are currently underway to assess the capacity of this tissue engineered periosteum to induce bone repair in an animal model.

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Harnessing Mechanical Cues to Enhance Cellular Migration in a Novel Tissue Engineered Dermal Substitute

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PURPOSE: Current dermal replacement products perform sub-optimally in complex wound beds, such as those that have been irradiated or those with exposed hardware, mostly as a result of insufficient cell invasion and vascularization. Angiogenesis is the result of multi-step processes which involve complex interactions between endothelial cells and their microenvironment. Cells sense the rigidity of their environment in a process called mechanotransduction, which is effected through integrin-mediated adhesions. Directional cell migration based upon substrate rigidity has previously been observed in a process termed durotaxis. We have fabricated a novel micropatterned microsphere scaffold (MSS) composed of differential densities of type I collagen in order to harness these signaling cues and promote rapid cell invasion and vascularization. Herein we compare the performance of MSS to a widely utilized, commercially available dermal replacement product (Integra®) in vitro and in vivo.

METHODS: Microspheres composed of 1% type I collagen 50-150um in diameter were created and encased in a 0.3% type I collagen bulk. For our in vitro study, polydimethylsiloxane (PDMS) wells of 4mm diameter and 2mm height were filled with the microsphere scaffolds. 3mm Integra® disks were placed inside PDMS wells. Non-microsphere containing 1% and 0.3% collagen scaffolds served as controls. A monolayer of human umbilical vein endothelial cells (HUVEC) was seeded onto this three-dimensional platform, stimulated with 1uM sphingosine-1-phosphate, and cultured for 3 days. The collagen hydrogels were then imaged using confocal microscopy and z-stacks obtained to quantify cell invasion. For the in vivo study, 8x2mm MSS disks were created, along with 1% and 0.3% collagen controls. 8mm Integra® disks were created, and the silicone layer was removed to allow invasion from either side (comparable to the other discs). A disk of each type was then implanted subcutaneously in the dorsum of 8-week old wild-type mice. The scaffolds were removed at 7 and 14 days, imaged, and analyzed with ImageJ.

RESULTS: In vitro results demonstrated significantly higher cell counts in both MSS and Integra® scaffolds compared to controls (p<0.001). Invading HUVEC penetrated significantly deeper in MSS compared to Integra® (mean depth of 73.5um vs. 40um, p<0.001), as well as 0.3% and 1% controls (mean depth of 13.4um and 12.2um respectively, p<0.001). In vivo results demonstrated robust cellular invasion throughout depth of the MSS construct, with more cells reaching the equator of the scaffold compared to Integra® and controls at both 7 days (p<0.05) and 14 days (p=0.03). Immunohistochemistry verified the presence of CD31 positive, CD45 negative cells within the MSS constructs.

CONCLUSION: These studies demonstrate superior cellular invasion of MSS both in vitro and in vivo compared to the current gold standard dermal regenerative template. Our novel hydrogel scaffold composed only of differential densities of type I collagen harnesses mechanical cues to significantly enhance cellular migration into the graft, offering a promising alternative to currently available dermal replacement products.

Minimizing Engineered Auricular Cartilage Contracture By Maximizing Construct/Cage Contact: The Importance of Injection Molding

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PURPOSE: As human auricular chondrocytes (HAuCs) remodel their environment and secrete extracellular matrix they exert intrinsic contractile forces. Previously we demonstrated that an external cage scaffold protected demolded chondrocyte-seeded type I collagen constructs from extrinsic compressive forces from the skin and soft tissue. We hypothesize that by allowing the HAuC-seeded collagen hydrogels to polymerize within cages via injection molding, the resulting construct will have increased surface area contact with the external scaffold, equating to a greater number of microscopic attachments between collagen polymers and the cage.

METHODS: Disc-shaped cages were designed using SolidWorks, then 3D-printed with polylactic acid on a MakerBot printer. HAuCs were harvested from discarded otoplasty remnants, then expanded to passage 3. HAuCs were mixed with10mg/mL type I collagen at 25million cells/mL. In group 1 a 2mm-high HAuC-seeded collagen sheet gel was allowed to polymerize for 30min under standard cell