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 1μM 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 with 10mg/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
culture conditions, then an 8mm biopsy punch was used to create 8mmX2mm discs that were then placed in well plates (“naked” (N) discs). In group 2, the same method was followed, but following punch biopsy, the discs were placed within individual cages before being moved to a well plate (“caged” (C)). In group 3, cages were placed within polydimethylsiloxane molds that contoured to the exterior of the cage, then HAuC-seeded collagen was injected directly into the cages and allowed to polymerize for 30 minutes under standard cell culture conditions before being moved to a well plate (“injection molded” (IM)). All groups were maintained with DMEM/F12 with 10% FBS and 1% Pen-Strep for 28 days. Groups were photographed on day 0 and day 28. Constructs underwent microCT for volume calculation on day 28. Images were analyzed in ImageJ.

RESULTS: On day 0, the average base areas of the 3 groups were compared (N: avg=49.6±2.5mm²) (C: avg=49.8±2.0mm²) (IM: avg=53.9±2.4mm²). Unpaired t-tests between groups showed significant area difference between N and IM (p=0.0233) and between C and IM (p=0.0192). On day 28, measurements were repeated (N: avg=36.6±7.5mm²) (C: avg=39.9±7.2mm²) (IM: avg=53.8±1.2mm²). Unpaired t-tests between groups showed significant area difference between N and IM (p<0.0001) and C and IM (p=0.0035). Constructs were imaged via microCT and volume was calculated on day 28 (N: avg=46.5±4.9mm³) (C: avg=51.4±1.6mm³) (IM: avg=123.7±22.9mm³). Unpaired t-tests between groups showed significant volume difference between N and IM (p=0.0001) and between C and IM (p=0.0001). Early evidence of auricular cartilage formation was seen histologically.

CONCLUSION: External scaffolding of “maturing” auricular cartilage results in significantly decreased loss of volume. Although PLA cages have been thought to confer protection against volume loss by shielding constructs from overlying compressive external forces, these in vitro studies demonstrate that it is the interaction between the hydrogel and the cage that is responsible for the minimal loss of volume observed in the injection molded group. Injection molding of HAuC-seeded collagen directly within our cages allows the hydrogel to polymerize and maximize attachment to the scaffold. This technique can be applied to in vivo constructs to develop mature elastic cartilage that maintains anatomically complex shapes.

Three-Dimensional Printing in Pediatric Medical and Surgical Applications - a Systematic Review

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BACKGROUND: Three-dimensional printing (3DP) technology is revolutionizing the medical field with applications ranging from surgical implants to patient education. This systematic review examines patient-specific applications of 3DP in the field of pediatrics, both medical and surgical.

METHODS: Terms related to “three-dimensional printing” and “pediatrics” were searched on PubMed, Scopus, Ovid MEDLINE, Cochrane CENTRAL, and Web of Science on January 14, 2018, returning 2122 unique articles. An initial title weed resulted in 819 abstracts for review. Inclusion and exclusion criteria concentrated on patient-specific pediatric applications of 3DP, yielding 367 articles for full-text review; 143 met all criteria for inclusion.

RESULTS: Two independent raters conducted an abstract weed (Cohen’s kappa = 0.78) and a full text weed (kappa = 0.96), yielding 143 studies with six unique pediatric, patient-specific applications of 3DP: pre-procedural planning (n=96), intraoperative use (n=49), patient education (n=8), medical team education (n=2), external devices including prosthetics and orthodontics (n=18), and tissue engineering (n=2). Thirty-five studies incorporated multiple 3DP applications; for example, 17 studies utilized 3DP in both pre-operative planning and intraoperative execution. Pre-procedural use (n=96) was further subdivided into three categories: planning (n=68), simulation (n=15), and pre-molding (n=13). Of the 175 total uses described in these 143 unique papers, 145 (82.6%) were related to preprocedural planning or operative use. 3DP was most commonly used in plastic surgery (n=33), dentistry (n=32), and cardiac surgery (n=23). Within plastic surgery, cranio/maxillofacial applications were most common, comprising 90.9% of studies. Studies reported variable approaches to manufacturing and utilized a wide range of printers and 3DP materials, reflected in the range of production time and costs. However, across studies, production trends emerged. Most commonly, CT was used for pre-operative analysis (n=87, 60.8%), Mimics was used for printer softwares (n=40, 28%), and Stratasys-brand 3D printer was used to manufacture 3DP products (n=20, 14%).