New York University School of Medicine, New York, NY, USA, Icahn School of Medicine at Mount Sinai, New York, NY, USA, Columbia University, New York, NY, USA, New York University College of Dentistry, Department of Biomatirials, New York, NY, USA, Baruch College, New York, NY, USA, New York University Langone Health, Hansjörg Wyss Department of Plastic Surgery, New York, NY, USA, New York University Langone Health, Department of Translational Medicine, New York, NY, USA

PURPOSE: The purpose of this study is to apply 3D-printed bioactive ceramic (3DPBC) scaffolds composed of beta-tricalcium phosphate (β-TCP) and coated in the osteogenic agent dipyridamole (DIPY) in a growing craniofacial animal model and: 1) quantify osteogenesis 2) assess suture patency. In our calvaria models, we further sought to identify the best scaffold design and dipyridamole concentration.

METHODS: In calvaria models, bilateral defects (10 mm) were created in 5-week-old New Zealand White rabbits (n = 16) 2mm posterior and lateral to the coronal and sagittal sutures, respectively. 3DPBC scaffolds were constructed in quadrant form composed of varying pore dimensions (220μm, 330μm, 500μm). Each scaffold was collagen coated and soaked in three concentrations of DIPY (100μM, 1,000μM, and 10,000μM) (n=8 each group). In cleft models, immature New Zealand White rabbits (n = 22) underwent unilateral 3.5mm by 3.5mm alveolar cleft defect injury. Defects were filled with 3DPBC scaffolds composed of 330μm pore size and soaked in varying concentrations of DIPY (4 in 100μM, 6 in 1,000μM, and 8 in 10,000μM). In both models, controls comprised of empty defects. All animals were euthanized 8-weeks post-operatively. Both models were analyzed using micro-computed tomography and histologic analysis. Mixed model analyses were conducted to compare pore size in the calvaria group and dosage effects on bone growth in both groups.

RESULTS: Scaffolds induced vascularized bone formation across the calvarial and cleft defects whereas control bone growth was restricted to margins in both. In calvaria models, dipyridamole concentration was analyzed independently of pore size to reveal that 1000μM resulted in the greatest degree of bone formation (p<0.05). Despite robust bone formation across all pores, there was no evidence for one size being significantly better than the other. In cleft models, 10,000μM resulted in greatest degree of bone formation (p<0.05). There was no exuberant bone formation across all concentrations of dipyridamole, and sutures remained patent in all experimental groups.

CONCLUSION: We present an optimized bone tissue engineering scaffold design and dipyridamole concentration for bone generation within growing pediatric calvarial and cleft defects which preserves cranial suture patency and does not form ectopic bone.

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Scaffolding the Scaffold: Mitigating Loss of Volume and Topography of Engineered Auricular Cartilage Using 3D Printed Contour Matching Cages

Alexandra Lin, BA1, Jaime Bernstein, BS1, Benjamin Cohen, BS2, Justin Buro, BA1, Karel-Bart Celie, BA1, Yoshiko Toyoda, BA1, Andrew Miller, BS1, Alice Harper, BA1, Lawrence J. Bonassar, PhD2, John P. Morgan, PhD1, Jason A. Spector, MD FACS1

1Laboratory of Bioregenerative Medicine and Surgery, New York, NY, USA, 2Meinig School of Biomedical Engineering, Ithaca, NY, USA

PURPOSE: Autologous reconstruction of the ear, whether for microtia or acquired deformity, is a complex procedure with substantial donor site morbidity and suboptimal aesthetic outcomes. An engineered auricular scaffold would obviate donor morbidity and provide improved aesthetic outcomes. A major obstacle to clinical translation of tissue-engineered auricles is the significant contraction and loss of topography that occurs during maturation of the soft collagen/chondrocyte matrix into elastic cartilage. Previously, we demonstrated that a 3D-printed biodegradable cage significantly mitigated contraction of simple disc-shaped collagen hydrogels seeded with human auricular chondrocytes.
(HAuCs) in vivo without impeding the development of elastic cartilage. Herein we fabricate cages to invest chondrocyte-collagen hydrogels with more intricate “anatomic” topographic features.

METHODS: Custom external cages were designed with a geometric element representative of the helical rim using SolidWorks (Dassault Systèmes, Vélizy-Villacoublay, France), then 3D-printed using polylactic acid (PLA) on a 5th generation MakerBot printer (MakerBot, New York, NY). Using auricular cartilage from discarded otoplasty specimens, HAuCs were harvested and expanded to passage 2. The chondrocytes were encapsulated into type I collagen hydrogels at a density of 25 million cells/mL with high fidelity contour matching to the cages. The hydrogels, either protected or unprotected by the PLA cages, were implanted into nude rats and explanted after 3 months.

RESULTS: After 3 months in vivo, all constructs developed a glossy white cartilaginous appearance, similar to native auricular cartilage. Histologic analysis demonstrated development of an organized perichondrium composed of collagen, a rich proteoglycan matrix, cellular lacunae, and a dense elastin fibrin network by safranin-O and Verhoeff’s stain. Biochemical analysis confirmed similar amounts of proteoglycan and hydroxyproline content in the constructs when compared to native auricular cartilage. Cage-protected constructs contracted significantly less than unprotected constructs on base area comparison (14.33% vs. 56%, p=0.0023), retained volume (213.4 mm³ vs. 117.2 mm³ compared to original volume of 280 mm³ and corresponding to 76.2% vs. 41.9% retention, p=0.0290), and maintenance of the topographic “helical rim” feature compared to unprotected constructs. Constructs were imaged via computed tomography with an Inveon Pre-clinical MicroPET/CT/SPECT (CTI/Siemens, Knoxville, TN), then digitally reconstructed with Imaris (Bitplane, Belfast, UK). Preservation of the “helical rim” feature was evaluated subjectively by gross examination and objectively by measuring the angle between the rim and base of the constructs, a measurement that demonstrated a significant difference between protected and unprotected constructs, respectively (151.8° vs. 197.7°, p=0.0445), and that indicated protected constructs better maintain the initial angle (110°) between rim and base.

CONCLUSIONS: We have shown that custom contour matched 3D-printed biocompatible/biodegradable external cages significantly mitigate contraction and maintain the complex topography of HAuC constructs. Furthermore, cages do not impede formation of mature elastic cartilage. This technique can be used to create custom cages that contour to any form, enabling the fabrication of engineered autologous cartilage tailored to individual patient anatomy, without the contraction and loss of topography that has thus far impeded translation to the clinic.

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A Novel Pharmaceutical Therapy Preserves Bone Cellularity in an Irradiated Model of Distraction Osteogenesis

Kevin M. Urlaub, BS, Jeremy V. Lynn, BS, Edward G. Carey, BS, Noah S. Nelson, MPH, Yekaterina Polyatskaya, MD, Alexis Donneys, MD, MS, Steven R. Buchman, MD

University of Michigan, Ann Arbor, MI, USA

PURPOSE: The use of distraction osteogenesis (DO) in craniofacial reconstruction is precluded by the deleterious effects of radiotherapy on bone and soft tissue, limiting reconstructive options for head and neck cancer (HNC) patients. Our research has previously demonstrated the individual efficacy of both radioprotective amifostine (AMF) and angiogenic deferoxamine (DFO) in improving healing metrics in irradiated models of mandibular DO. Through histologic evaluation, this study investigates the synergistic effects of AMF and DFO as a novel combination therapy in order to expand the utility of DO as a reconstructive technique for HNC patients following radiotherapy (XRT).

METHODS: 30 male Sprague Dawley rats were divided into five groups: DO, XDO, AMF, DFO, and Combined Therapy (CT). With the exception of the DO group, all rats were administered a fractionated, human-equivalent radiation dose of 35 Gy, comparable to 70 Gy administered to HNC patients clinically. All groups underwent mandibular osteotomy and placement of an external fixator device. Beginning on post-operative day 4, the left hemi-mandible was distracted over the course of 8 days to a critical-sized defect of 5.1 mm. All rats were sacrificed on post-operative...