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3D-Printed Bioactive Ceramic Scaffolds for Induction of Osteogenesis in the Immature Skeleton

Presenter: Samantha G. Maliha, BA

Co-Authors: Madison E. Cox, HSD; Juliana Gomez, BA; Sejndi Rusi, BA; Alan Meskin, BA; Jonathan M. Bekisz, BA; Christopher D. Lopez, BA; Lukasz Witek, MSci, PhD; Paulo G. Coelho, DDS, PhD; Roberto L. Flores, MD

Affiliation: New York University Langone Health, New York City, NY

BACKGROUND/PURPOSE: 3D-printed bioactive ceramic (3DPBC) scaffolds composed of beta-tricalcium phosphate (β-TCP) and coated in the osteogenic agent dipyridamole (DIPY) have been previously shown to heal critically sized calvarial defects in several adult animal models.1-3 This bone tissue engineering construct has yet to be applied in a pediatric craniofacial model where the pathologic effects of osteogenic agents on the growing sutures may be of concern. The purpose of this study is to apply the described bone tissue engineering construct in a pediatric growing animal model and 1) quantify osteogenic potential in a growing calvarium; 2) maximize the scaffold design and dipyridamole concentration for the growing calvarium; and 3) characterize the effects of this bone tissue engineering construct on the growing suture.

METHODS/DESCRIPTION: Bilateral calvarial defects (10mm) were created in 5-week-old New Zealand White rabbits (n = 14) 2mm posterior and lateral to the coronal suture and sagittal sutures, respectively. 3DPBC scaffolds were constructed in quadrant form composed varying pore dimensions (220μm, 330μm, 500μm). Each scaffold was collagen coated and soaked in varying concentrations of DIPY (100μM, 1000μM, 10,000μM). Controls comprised empty defects and collagen coated scaffolds. Scaffolds were then placed into the calvarial defects to fill the bone space. Animals were euthanized 8-weeks post-operatively. Calvaria were analyzed using micro-computed tomography and 3D reconstruction. Mixed model analyses were conducted considering pore size and dosage effects on bone growth (α=0.05).

RESULTS: 3DPBC scaffolds generated bone throughout the construct (defect marginal and central regions) while bone healing in empty sites was restricted to the defect margins confirming its critical size dimension at 8 weeks in vivo. When evaluating volume occupied by bone solely as factor of pore size (small, medium, and large), the small pores yielded the highest mean value (26.8% ± 3.4) when compared to medium and large. However, analysis indicated no statistical differences between the sizes (p>0.10). In assessing the effectiveness of coating the scaffold in either collagen or dipyridamole (DIPY), higher mean bone occupancy values were observed in the scaffolds coated in 1,000μM DIPY (27.9% ± 4.05), which was significantly greater in comparison to the collagen-coated scaffolds (20.9% ± 4.43, p=0.021). Growing cranial sutures remained patent across all concentrations of DIPY, including 10,000μM.

CONCLUSION: 3DPBC scaffolds effectively generates bone in a growing calvarial animal model. Pore size and dipyridamole dose has been optimized for the growing cranium. Cranial suture patency is preserved even at a 2 log increase over the effective ostetogenic dose.

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Osteoprotegerin-Mediated Osteoclast Inhibition Is Augmented on Nanoparticulate Mineralized Collagen Glycosaminoglycan Materials

Presenter: Justine C. Lee, MD, PhD
Co-Authors: Xiaoyan Ren, MD, PhD; David Foulad, MD; David Bischoff, PhD; Timothy A. Miller, MD; Dean Yamaguchi, MD, PhD; Brendan A. Harley, ScD

Affiliation: David Geffen School of Medicine at UCLA, Los Angeles, CA

PURPOSE: Coordination of bone formation and resorption is necessary for the success of bone regenerative strategies. Characterization of the instructive capabilities of extracellular matrix (ECM)-inspired materials for osteoprogenitor differentiation has sparked questions on the interactions between such materials and the host microenvironment. Previously, we demonstrated that a nanoparticulate mineralized collagen glycosaminoglycan (MC-GAG) scaffold is both highly osteogenic without the need for exogenous growth factor stimulation and induces secretion of osteoprotegerin (OPG), an endogenous decoy receptor against the receptor activator of nuclear factor-κB ligand (RANKL), a necessary osteoclastogenic factor. In this work, we combine an adenoviral mediated expression of OPG (AdOPG) in primary human mesenchymal stem cells (hMSCs) with MC-GAG to understand the role for osteoclast inactivation in augmentation of bone regeneration.

METHODS: Control and AdOPG transduced primary hMSCs were cultured on Col-GAG or MC-GAG materials in osteogenic differentiation medium. OPG and RANKL expression were evaluated using quantitative reverse transcriptase polymerase chain reaction (QPCR), western blot analysis, and enzyme linked immunosorbent assay (ELISA). Co-cultures of control and AdOPG transduced hMSCs on Col-GAG and MC-GAG with primary human osteoclasts were performed. Osteogenic differentiation was evaluated with western blot, ELISA, and micro-computed tomography for osteogenic differentiation. Simultaneously osteoclast activity was assessed with tartrate resistant acid phosphatase staining and resorption pit assays.

RESULTS: hMSCs differentiated on MC-GAG expressed a lower ratio of endogenous RANKL/OPG protein compared to a non-mineralized collagen glycosaminoglycan (Col-GAG) scaffold. In both materials, the RANKL/OPG ratio was further lowered significantly in the presence of AdOPG compared to control hMSCs without significant difference between the materials. We established a co-culture system to understand the interplay between differentiating hMSCs on Col-GAG or MC-GAG and differentiating human primary pre-osteoclasts. Control hMSCs on Col-GAG or MC-GAG in co-cultures did not differ in viability or proliferation compared to each other or to hMSC single cultures. However, AdOPG-transduced hMSCs on MC-GAG was modestly decreased in viability or proliferation compared to the Col-GAG counterpart in co-cultures. Co-cultures with differentiating osteoclasts increased hMSC mineralization with or without AdOPG, particularly in MC-GAG. In contrast, the viability and proliferation of osteoclasts in co-culture were significantly decreased in the presence of AdOPG-transduced hMSCs. While co-culture with control hMSCs on either Col-GAG or MC-GAG upregulated the resorptive activity of osteoclasts, AdOPG-transduced hMSCs reduced the resorption with a greater effect on MC-GAG compared to Col-GAG.

CONCLUSION: The addition of osteoprotegerin to MC-GAG-mediated hMSC osteogenic differentiation simultaneously diminishes osteoclast resorptive capacity without affecting the positive regulatory effects on osteogenic differentiation.

Inferring 3D Craniofacial Skeletal Shape from Facial Surface Geometry Using Reverse Engineering of a Forensic Tissue Depth Model

Presenter: Zachary Fishman, P.Eng, MASc

Co-Authors: Oleh Antonysyhyn, MD, FRCS(C); Jeffrey A. Fialkov, MD, MSc, FRCS(C); Cari Whyne, PhD

Affiliation: University of Toronto & Sunnybrook Research Institute, Toronto, ON

PURPOSE: The face and craniofacial skeleton (CFS) make up a complex 3D structure that is critical to human function and cosmesis. Traumatic injury to the CFS requires fracture treatment to both allow the recovery of mechanical function and forms a foundation for the restoration of soft tissue anatomy. CFS reconstruction aims to restore pre-injury appearance, however in severe injuries shape information for the skull and facial bones may be missing. This presents a particular challenge in bi-frontal injuries and pan-facial fractures where the mirror imaging of the intact side of the head cannot be used to guide reconstruction.