Osteoclast Activity Required For Cranial Suture Patency

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PURPOSE: Zebrafish continually produce new bone material, reshape it, and repair it as needed to sustain lifetime growth and skeletal integrity. This is accomplished by synchronized efforts of osteogenic cells such as osteoblasts, osteocytes, and osteoclasts. The elastic joints formed between flat bones of the cranium called sutures provide adaptation, which can be compromised by suture obliteration and bone fusion associated with disorders such as craniosynostosis. Here, we describe the genetic and histological study of the zebrafish koliber mutant, which presents with sporadically fused frontal bones. The koliber mutation has a regulatory character with recessive, Mendelian inheritance. During embryonic development, heterozygotes and homozygotes are undistinguished phenotypically from the wild type siblings. Morphological changes, such as the shortening of body length and a noticeably rounded head, become visible at the larval stage. The mutant abnormalities advance with age significantly, though variations of the phenotype are typical for this mutation. Simultaneous staining for bone and cartilage revealed progressive loss of cartilage, severe compression of vertebral column, fusions of vertebrae, and sporadically fused frontal bones. We hypothesize that the koliber phenotype results from a functional imbalance between osteogenic cells.

METHODS: RNAscope in situ hybridization on paraffin sections was used to analyze genes’ expression pattern. Histological and TRAP staining for osteoclast activity was also performed on paraffin sections. The qRT-PCR was used to verify genes expression level. To analyze the cellular organization of the sutural tissue, we implemented TEM. The osteogenic cells were statistically evaluated based on their position within the sutural tissue. All results were concluded based on a minimum of three independent experiments. Lurie Children’s IACUC committee approved zebrafish husbandry and experimental methods.

RESULTS: Statistical analysis applied to histologically stained specimens revealed significantly more cells attached to the frontal bone of the mutant than wild type (72.7%) and 64.5% respectively (p = 0.02). RNAscope in situ identified these cells as terminally differentiated osteoblasts positive for bglap, suggesting intensive bone matrix synthesis. These results were supported by TEM evaluation, which additionally exposed more complex, collagen-rich ECM in the mutant. Concurrently, we observed a prominent reduction (by 50%) of acp5a transcription, the gene encoding TRAP activity, indicating lower activity of osteoclasts in the mutant. This was further confirmed by histological staining for TRAP enzyme. The qRT-PCR analysis for genes regulating osteogenic cells function revealed a significant deficiency in the transcription of genes required for osteoclasts’ maturation but not for the development of their precursors. In addition, we observed increased expression of osteocyte markers such as phex and spp1.

CONCLUSION: Our results suggest an inadequate balance between osteogenic cells, with more osteoid synthesis and a simultaneous deficiency in bone remodeling in the mutant. We propose that the excessive/irregular frontal bone growth observed sporadically in the wild type is normally corrected by recruited osteoclasts. In contrast, in the koliber mutant reduced osteoclast activity interferes with this functionality, resulting in bone fusion.

Bone Tissue Engineering of the Pediatric Calvarium and Alveolus using Dipyridamole-coated 3D-Printed Bioactive Ceramic Scaffolds

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**PURPOSE:** The purpose of this study is to apply 3D-printed bioactive ceramic (3DPBC) scaffolds composed of beta-tricalcium phosphate ($\beta$-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**

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**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.