Modulating Phosphate Metabolism for Periodontal Regeneration

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Statement of Purpose: Phosphate (P_i) and pyrophosphate (PP_i) levels are essential for normal development of mineralized tissues. P_i compounds with calcium ions to form hydroxyapatite, while PP_i is a potent inhibitor of hydroxyapatite mineral growth. Physiological mineralization and ectopic calcification is influenced by the PP_i /P_i ratio [1]. Local concentration of PP_i in the extracellular space is controlled by enzymes and transporters including tissue nonspecific alkaline phosphatase (TNAP), which reduces tissue PP_i and generates P_i, and factors increasing extracellular PP_i, e.g. progressive ankylosis protein (ANK), and ectonucleotide pyrophosphate phosphodiesterase 1 (NPP1). We characterized developmental consequences of PP_i and P_i dysregulation on tooth root development. Based on our and others' observations that altered PP_i causes marked cementum phenotypes [2, 3], we hypothesized that regulation of PP_i/P_i levels might encourage cementum repair/regeneration. To test our hypothesis, we used a mouse calvarial defect model.

Methods: Tooth root development of mutant and knock-out (KO) mouse models with altered P_i or PP_i metabolism was characterized by histology, immunohistochemistry, *in situ* hybridization, electron microscopy, and nanoindentation. We employed a method to immobilize TNAP (ALP) on microporous nanofibrous fibrin scaffolds (FS). FS were fabricated using sphere-templating methods. ALP was covalently immobilized on FS using 1-EDC [4]. Critical size defects (5mm) were made in calvaria of 5 wk old mice (n=12). Defects were treated with FS alone, ALP/FS, or left empty, then examined by histology and microCT 45days following surgery.

Results: Cementum was most profoundly affected by alterations in local PP_i levels, while altered P_i levels led to more subtle cementum phenotypes [5, 6]. Decreased PP_i (decreased PP_i/P_i), as in the *Ank* KO mouse, increased cementum formation (Fig. 1). In an attempt to recapitulate developmental conditions encouraging cementogenesis, we added ALP/FS to calvarial defects to modulate local PP_i/P_i. After 45 days, the immobilized rhALP/FS resulted in greater bone formation within the defect area compared to empty defect control (Fig. 2).

Conclusion: Cementum is sensitive to PP_i dysregulation (Fig. 3). Immobilized ALP/FS and other biomaterials approaches to modulate PP_i/P_i are candidates for regenerating cementum, bone, and other mineralized tissues.

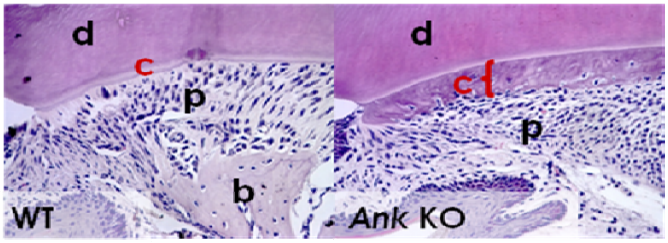

Fig. 1. Histological analysis of WT and *Ank* KO molar at 45dpc. d: dentin, c: cementum, p: periodontal ligament, b: alveolar bone.

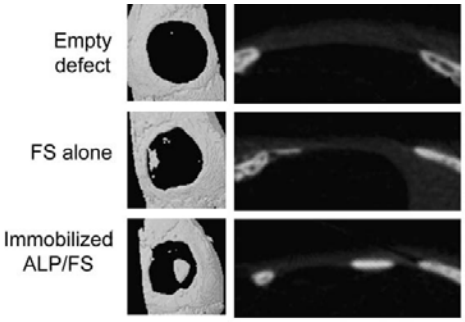

Fig. 2. Representative micro CT images for mouse calvarial defects 45 days after surgery[4].

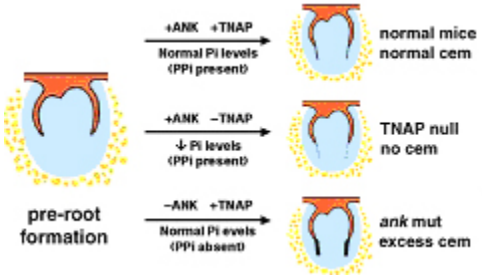

Fig. 3. PP_i/P_i ratio regulates cementum formation [2].

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