Amelogenin-mediated Odontogenesis and Osteogenesis: Signaling Pathway and Therapeutic Potential
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Introduction
Amelogenin is a protein product of ameloblasts in enamel formation and critical to the structure and mineralization of enamel in development. Amelogenin isoforms comprise ~90% of the mineralized matrix that covers the crown of the tooth bud. As amelogenin is cleaved and degraded, mineral deposition in the form of crystals takes place in a hierarchical pattern. During amelogenesis, an organic, protein-rich substance which comprises over 85% amelogenin is transformed into a completely mineralized architecture of hydroxyapatite of enamel. In the present study, we take advantage of the remarkable mineralization potential of amelogenin, and apply this potential in a therapeutically consistent model to promote odontogenesis and osteogenesis in tissue regeneration.

Methods
Expression and purification of recombinant protein
We first cloned recombinant mouse amelogenin (rM179) into expression vector, pDEST17 and verified amelogenin presence by sequencing using M13 primers. For expression in Escherichia coli (E. coli), the expression clone in pDEST17 containing the T7 promoter was transformed into a BL21(DE3)pLysS. E. coli were grown in LB medium until the optical density (OD600) of the culture reached 0.4–0.5. A final concentration of 0.7 mM isopropylthio-b-D-galactoside (IPTG) was added to induce the expression of rM179. The cells were harvested and resuspended in a bacterial protein extraction reagent with 8M guanidine chloride. After centrifuge, the supernatants were applied to a Cobalt Sepharose 6 resin column. After elution with 0.5M EDTA, the protein was dialyzed with PBS containing 10% glycerol. The purity of recombinant amelogenin was determined by SDS-PAGE gel followed by Commassie blue staining.

Isolation and Amelogenin-mediated Odontogenesis and Osteogenesis of Dental Stem Cells
Exfoliating deciduous incisors and permanent third molars of multiple donors were collected with IRB approval. Dental pulp (DP) and periodontal ligament (PDL) cells were isolated and enzyme-digested per our prior methods (1). Mononucleated and adherent cells were cultured in DMEM-LG medium containing 10% FBS and 1% antibiotics in 10 cm culture dishes. Per our prior data (1), these isolated cells contain single clones of multipotent cells that readily express stemness markers such as Stro-1, Oct4, Nanog and CD146. DPCs and PDL cells were expanded and subjected to osteogenic and odontogenic differentiation. Briefly, DP and PDL cells were cultured in DMEM-LG medium containing 10% FBS, 50μg/ml ascorbic acid and 10mM 2-glycerophosphate in the presence or absence of recombinant amelogenin. The osteogenic and odontogenic differentiation was determined by the expression of RUNX2, dentin sialophosphoprotein (DSP) and dentin matrix protein-1 (DMP-1) by real-time PCR, as well as Von Kossa and alkaline phosphatase staining. The level of active β-catenin was measured by immune-florescence staining and activation using an antibody against the active (non-phosphorylated) form. Amelogenin treated PDL and DP stem cells showed β-catenin nuclear translocation and accumulation of non-phosphorylated β-catenin.

Results
Amelogenin treated DP and PDL stem cells showed robust ALP and Von Kossa staining in comparison to untreated controls upon 2-wk culture (Fig. 1A). Despite similar RUNX2 expression, amelogenin-treated DP and PDL cells expressed DSP, which was absent without amelogenin treatment (Fig. 1B).

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References
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Disclosure
The authors have nothing to disclose.