A Synthetic C-terminal Human Amelogenin Fragment as a Viable EMD Alternative for Periodontal Regeneration

Purpose of the Research: Emdogain (EMD, Struamann) is an FDA-approved porcine fetal enamel matrix mixture for periodontal regeneration. Previous studies have revealed that Amelogenin (AMEL) proteins are the major component of EMD (>90%). However, due to its complex nature as a natural enamel matrix derivative, the precise protein composition of EMD and the mechanism of its effects on surrounding tissues are unknown. To identify a cost-effective and well-defined alternative to EMD, we asked whether amelogenin fragments alone and in absence of non-defined porcine matrix components will mimic or surpass EMD biological and regenerative properties in regards to periodontal wound healing and alveolar bone regeneration.

Methods and Materials: Emdogain (EMD) was compared with recombinant human AMEL isoforms rh174, rh163 and rh146 and synthetic amelogenin peptides sh147-163 and sh164-174. For *in vitro* studies, human periodontal ligament (PDL) cells were treated with hAMEL proteins, or peptides, or EMD at a concentration of 10 μg/ml for 3, 7, 14 and 21 days. Functional assays included BrdU incorporation for cell proliferation, a scratched wound healing assay for cell migration, and alkaline phosphatase (ALP) and Alizarin Red staining for cell differentiation. The effect of hAMELs and EMD on gene expression was determined using Reverse Transcription Polymerase Chain Reactions (RT-PCR) and Western blotting. For *in vivo* studies, collagen sponges (COL) were coated with human AMELs and EMD at a concentration of 10 μg/ml and then lyophilized. The AMEL-COL or EMD-COL constructs were implanted into C57 mouse skin subcutis or alveolar bone defects, dissected and processed for fixation or freezing after 4 or 8 weeks. Samples were processed for μCT, histology, immunohistochemistry, Western blotting and/or RT-PCR.

Results: rhAMEL isoforms rh174 and rh163 from the amelogenin C-terminus increased the number of cells positive for BrdU incorporation compared to the rh146 and control groups, respectively. In addition, cell scratch assays demonstrated that rh174 and rh163 promoted cell migration across the gaps and closed

the wound on day 4, one day earlier than in the rh146 and BSA treatment groups. However, RT-PCR analysis revealed that rh163 downregulated Col 1 and 3 gene expression and inhibited ALP activity while rh146 increased ALP activity in PDL cells. The effects of the shAMEL164-174 peptide on PDL cell behavior was similar to EMD, including enhanced PDL cell migration, increased proliferation, enhanced ALP activity and augmented mineral nodule formation. H&E and Masson staining demonstrated that local MSCs migrated into the sh164-174/Col and EMD/Col subcutaneous implants after 4 weeks. Eight weeks after implantation, MSCs resided within sh164-174/Col and EMD/Col scaffolds and were surrounded by new extracellular matrix, while the sh146-163/Col and BSA/Col implants revealed only isolated individual cells at the outer implant margins. MicroCT analysis indicated that the ratio of bone volume to tissue volume was 0.34 in periodontitis control group, 0.42 in sh146-163 treatment group, 0.44 in sh164-174 group and 0.32 in the EMD group, respectively. The bone density was sh164-174> EMD>sh146-163/Col>control. The differences in bone density were further confirmed by Mason trichrome staining, revealing evidence of mature bone formation only in the shAMEL164-174 treatment group. In addition, H&E staining demonstrated that sh164-174 treatment significantly promoted periodontal ligament reattachment to the tooth root, while the PDL remained delaminated in the EMD, sh146-163 and control groups.

Discussion: Enamel matrix-derived EMD is a widely used biomimetic for the treatment of periodontal defects. Our studies demonstrated that in comparison to EMD, shAMEL164-174 exhibited similar or superior effects on periodontal soft tissue wound healing and alveolar bene regeneration. Benefits of the synthetic shAMEL164-174 are its cost effectiveness and purity, devoid of the non-defined pig enamel matrix components present in EMD. When applied to periodontal defects in periodontitis animal models, shAMEL164-174 recruited mesenchymal stem cells, promoted periodontal ligament re-attachment and enhanced alveolar bone regeneration.

Conclusion: The small amelogenin peptide shAMEL164-174 is a cost-effective alternative to EMD with added benefits to patients concerned about xenografts and/or ill-defined porcine matrix contaminants.