Dental Pulp Regeneration Using Novel Self-Assembling Peptides

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***Abstract*—Dental pulp regeneration is a sought after alternative to root canal procedures and fillings which result in devitalized teeth. This project aims to develop an injectable biocompatible multidomain peptide (MDP) hydrogel scaffold that promotes dental pulp regeneration and will create a revitalized tooth *in vivo*.**

INTRODUCTION

Tooth caries (decay) pose a substantial public health concern affecting children and adults. Over 15 million root canals are performed annually in the US costing ~$1000/procedure. Over 92% of adults 20 to 64 have had at least one in their permanent teeth. [1] There exists no FDA approved therapy for regeneration post-partial pulpotomy. Current standard of treatment for dental caries is to remove diseased pulp and replace it with GuttaPercha or composite materials, which ultimately results in non-responsive "dead teeth" that demineralizes, losing structural, functional, and sensory integrity. They are prone to reinfection and fracture. We will deliver a pro-blood vessel forming, dental pulp regenerating hydrogel to regrow pulp after a partial pulpotomy. The promise to replace outmoded rubber inserts in living pulp cavities with vital pulp is in principle a major step forward for dental tissue engineering.

Research Significance

Pulp regeneration post extirpation is thought to be the Holy Grail of regenerative endodontics. Beginning with formation of a fibrin scaffold with entrapped platelets and red blood cells, unguided regeneration of periapical tissue (bone, ligament, or cementum), distinctly different from pulp, was achieved over 50 years ago. [2]

Multidomain peptides (MDP) are short amino acids sequences with repeating hydrophobic and hydrophilic motifs that can be triggered to self-assemble in aqueous solution to form β-sheets and long-range nanofibers. [2] Self-assembly is mediated by non-covalent bonds that break and reassemble quickly: hydrogen bonding, Van der Waal's interactions, and ionic interactions.

This affords thixotropic rheological properties - rapid shear thinning and shear recovery. [2] Therefore, these hydrogels can be easily syringe aspirated, injected, and re-assemble *in situ* to provide a prolonged, sustained response, which has been evaluated for drug delivery, angiogenesis, inflammation modulation, and recovery from ischemic tissue disease. [2-5]

Project Overview

To achieve functional vital pulp responsive to the oral cavity and gingiva, a partial pulpotomy followed by hemorrhaging or placement of biological scaffolds (fibrin and the like) has not resulted in adequate pulp regrowth. Without the instruction for angiogenesis and dentinogenesis, current scaffolds lack in translatability. In this proposal, we explore how extracellular matrix (ECM) mimetic scaffolds present a method to promote rapid cellular infiltration, angiogenesis, dentinogenesis, and regeneration of functional dental tissue.

Our central hypothesis is that an ECM mimetic hydrogel scaffold, modified with dentin and/or angiogenic domains and coupled with growth factor delivery, will modulate the dentinogenic and angiogenic responses to one of healing and resolution. Ultimately, this hydrogel will become a supportive environment for enhanced natural tissue revitalization of pulp *in vivo*.

We propose the use of MDP, which are easily tailorable with growth factor mimics and capable of loading growth factors, cytokines, and/or cells. MDP are short amino acid sequences with repeating hydrophobic and hydrophilic motifs that can be triggered to self-assemble in aqueous solution to form β-sheets and long-range nanofibers. [2] Self-assembly is mediated by non-covalent bonds that break and reassemble quickly: hydrogen bonding, Van der Waal's interactions, and ionic interactions.

At the ultrastructural level, MDP self-assembles into large-scale ECM mimetic nanofibers 2nm thick, 6nm wide, and nm to μm long. Injectable ECM mimetic scaffolds may rapidly infiltrate with cells that loaded drug can phenotypically modulate. *In vitro* data has shown evidence of key criteria vital to the objectives of the proposed work: 1) generation of biomimetic nanofibers, 2) generation of non-degradable and degradable constructs, 3) ability to deliver materials through small bore needles, and 4) controlled release of growth factors. [3, 6] The purpose of our study is to improve the current standard of care by generating scaffolds that promote healing and regeneration to ultimately regenerate vitalized pulp rather than replace it with non-biological media. Regeneration of vital pulp will allow dynamic response and defense against future carious insults.

Variants of peptides based on the sequence $K_2(SL)_6K_2$ can be tailored to stimulate dentinogenesis and angiogenesis both *in vitro* and *in vivo*. Injectable, *in situ*, self-assembling scaffolds will be implanted in partially extirpated pulps in rodent and canine models of carious insult. Overall, this proposal will aim to develop procedures for drug delivery utilizing: i) a self-assembling MDP synthesized with a potent promoter of angiogenesis (QK) ii) to deliver MEPE with initial *in vitro* optimization and iii) subsequent implantation *in vivo* into partially extirpated pulp models.

The hypothesis is that regulation of the dentinogenic and angiogenic responses may allow a partial pulpotomy and hydrogel delivery with subsequent regeneration of native pulp to become a mainstay of endodontic care. While showcasing guided dental pulp regeneration via guided angiogenesis and dentinogenesis, the library of materials created in this proposal

can be tailored to a variety of applications in soft tissue and bone tissue engineering and translational medicine.

Specific Aims

Specific Aim 1

Design and characterization of functional MDP that promote tissue regeneration with tailorable drug release is the primary goal of the project (Figure 1).

MDP with a base structure of $K_2(SL)_6K_2$- will be modified with i) a dentin promoting matrix extracellular phosphoglycoprotein (MEPE) mimic (also called dentonin) to promote dental stem cell (DPSC) proliferation or ii) a angiogenic vascular endothelial growth factor (VEGF)-165 mimic (QK) to promote vascularization. Concomitant release of mimics from hydrogel scaffolds will assay efficacy of a conjugation or drug delivery strategy. Specifically, two areas will be investigated: 1) optimization of MDP for nanofibrous hydrogelation and 2) temporal control of drug release. Success will be measured as a function of synthetic matrix stability *in vitro* and time controlled release of growth factors and cytokines as a function of matrix loading and composition.

Specific Aim 2

Preliminary *in vitro* and *in vivo* response to MDP composition follows the design and production of the MDP. These novel MDP will be investigated for determination of cytocompatibility with fibroblasts, dental pulp stem cells, and polarization of macrophages. Optimized scaffolds will be evaluated for preliminary *in vivo* biocompatibility in a rat dorsal subcutaneous model. Success criteria for material combinations include: 1) no cytotoxicity, 2) evaluation of cytokine utility in modulating inflammatory cell phenotype, 3) development of angiogenic networks, and 4) effective proliferation, migration, and differentiation of dental stem cells.

Specific Aim 3

Recapitulation of a niche for dental pulp regeneration subsequent to partial pulpotomy *in vivo*. A partial pulpotomy model will be employed in rat molars with subsequent injection of MDP; this will be used to evaluate efficacy. Subsequently, success criteria driven biocompatible scaffolds will be evaluated in a dog partial pulpotomy model. Both *in vivo* partial pulpotomy models will utilize pre-molar surgical cut-down, management of bleeding and subsequent injection of *in situ* self-assembling hydrogel. Specifically, three areas will be investigated: 1) immune cell response, 2) recruitment and polarization of inflammatory cells, and 3) regeneration of native tissue and dental pulp.

Expected Outcomes

We will develop an angiogenic and dentinogenic MDP that will promote DPSC viability and proliferation *in vitro* and have controlled drug release of cytokines and growth factors. Promising hydrogels will be cytocompatible, modulate inflammatory cell phenotypes, and develop angiogenic networks. This peptide, when injected into a rat partial pulpotomy model *in vivo*, will display proliferation of dental cells, resulting in a regeneration of the pulp tissue with adequate blood supply and no inflammatory immune response. Furthermore, the repair and regeneration of dentin layer will be observed, resulting in the restoration of the dentin-pulp complex. This will be exhibited in both rat and dog pulpotomy models.

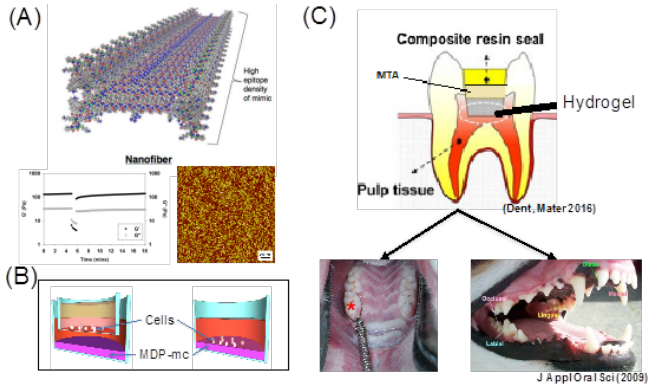

Figure 1. (A) MDP ± drug will be designed, synthesized, and characterized. An AFM image shows nanofibrous structure and the rheology data demonstrates its thixotropy (needle injectability). (B) Cytocompatibility will be evaluated in 2D and 3D cultures. (C) Scaffolds will be optimized and loaded into partial pulpotomies created in the first maxillary molars in rodents (*) and molars in canines.

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