

PDGF Gene Therapy to Promote Oral Implant Osseointegration

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Background and Introduction: Tooth loss is a common consequence of oral disease or injury to the craniofacial complex. Over 240 million people in the industrialized world are missing one or more teeth. Of these, only 2% receive tooth replacement dental implants. Despite the costs associated with oral implants, tooth replacement sales are growing at a year-over-year rate of 15-18%, and are estimated to exceed \$1.1 billion USD globally, thus representing a significant health care burden. Tooth loss can lead to the destruction of nearly half of the original tooth-supporting (or alveolar) bone¹. Traditional techniques for enhancing bone formation for oral implant placement include bone autografts, allografts or guided bone regeneration². The use of osteogenic growth factors such as platelet derived growth factor (PDGF) to regenerate tooth-supporting and peri-implant alveolar bone in preclinical animal models^{3,4,5,6,7} and in early human trials^{8,9} offers significant potential for periodontal regenerative medicine. However, outcomes of these therapies are limited in terms of regeneration and predictability, in part due to drug instability at the site of delivery. Therefore, the utilization of gene therapy to control the release and bioavailability of osteogenic GFs offers potential for tissue engineering of osseous defects¹⁰.

Recently, our group has demonstrated the potential of using gene delivery to regenerate alveolar bone and cementum around teeth and alveolar bone associated with dental implant fixtures^{11,12} (Jin, et al. 2004; Dunn, et al. 2005). These studies have demonstrated strong potential for the use of gene therapy for bone regeneration.

Delivery of PDGF by gene transfer has been shown to stimulate gingival fibroblast, PDL and tooth-lining cell (cementoblast) mitogenesis and proliferation above that of continuous PDGF administration in vitro^{13,14}. PDGF has also demonstrated positive effects in regenerating bone around teeth and dental implants. The primary goal of this application is to validate novel PDGF gene delivery regenerative medicine strategies and

apply them to animal models with a long-term goal of human application.

Rationale: For gene therapy to become a clinical reality for human application in the treatment of disease or injury, safety must be a primary concern. The use of viral vectors for growth factor delivery to bone defects requires evaluation of specific vector biodistribution properties (i.e., dissemination of vectors from the osseous site to other extraorthopic tissues and organs). Various safety assessments have been performed using growth factor transgenes¹⁵ and for bone-sparing agents preclinically¹⁶ demonstrating lack of significant local and systemic toxicity. The continued diligence in carefully evaluating both short-term and long-term safety of gene therapy vectors will be important if gene therapy is to become a viable treatment alternative for bone repair applications. Thus, the major goal of this application is to determine the potential of PDGF gene delivery to repair alveolar bone defects and to comprehensively assess safety in an attempt to grow bone where success has not been met using traditional growth factor application.

Methods: The maxillary first molars of male Sprague-Dawley rats were extracted bilaterally and the extraction sockets and soft tissues were allowed to heal for 30 days. Osteotomy defects were subsequently created and 1 x 2 mm titanium oral implants were then press fit into position (Figure 1). The remaining surface of the implants and the osseous defects received the following treatments: adenovirus encoding PDGF-B (AdPGFB 8 E11 particles/ml) in 2.6% collagen gel (n = 8) or collagen alone (n = 8). In an alveolar defect model utilizing AdPDGFB virus, viral copies within the blood and organs were detected using real time PCR in all of four groups: high dose Ad-PDGFB (8 E11 particles/ml), low dose Ad-PDGFB (8 E10 particles/ml), collagen alone, and untreated control. In addition, biodistribution of the virus was evaluated using in vivo bioluminescence using Ad-

luc as a reporter to evaluate dissemination of the vectors beyond the craniofacial complex.

Results: By four weeks post-surgery, both the AdPDGFB and collagen only groups demonstrated more mineralized tissue when compared to the two week time points ($p < 0.05$). Viral copies detected in the blood were not significantly different between treated and untreated rats at all time points. Viral copies within the organs were also not significantly different between treated and untreated rats for all time points except for a slightly elevated level found in the high-dose group at 14 days post surgery in the liver and spleen. Bioluminescence results demonstrated the localization of the vector to the defect site, with minimal dissemination to the organs over the course of 70 days. Finally, from the set of preliminary micro-CT images, representative images were converted to a 3D finite element model for simulated biomechanical testing.

Discussion and Conclusion: These preliminary and ongoing data suggest the feasibility of administering and targeting PDGF gene therapy vectors to oral implant defects and the ability of this model to be assessed in terms of efficacy and safety. Finally, these data confirm the potential of CT imaging of titanium implants for quantification of alveolar bone and simulated biomechanical testing.

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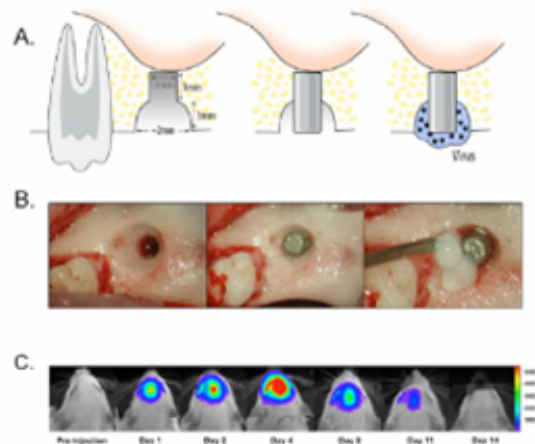


Figure 1: A. Oral Implant Osteotomy Defect Model for PDGF Gene Delivery. “Well-type” osteotomy defects B. High magnification photos from the surgical operation including defect creation (left panel), dental implant placement (middle) and gene delivery (right). C. Optical imaging of reporter gene, Ad-luciferase targeting to oral implant defects. Color enhancement demonstrates localization of luciferase protein production following injection of luciferin substrate into animals from baseline to 14 days post-gene delivery to oral implant defect sites.