Evaluation of an Injectable Keratin Biomaterial for Delivery of rhBMP-2 in a Porcine Mandible Defect

Seth Tomblyn, PhD\(^1\), Justin M. Saul, Ph.D.\(^2\), Pamela Brown Baer, DDS MEd\(^3\), David Silliman\(^4\), Eric Vanderploeg, PhD\(^5\), Thomas L. Smith, PhD\(^6\), Luke Burnett, PhD\(^1\).

\(^1\)KeraNetics, LLC, Winston-Salem, NC, USA, \(^2\)Dept of Chemical, Paper, and Biomedical Engineering, Miami University, Oxford, OH, USA, \(^3\)US Army Institute of Surgical Research, Ft Sam Houston, TX, USA, \(^4\)US Army Institute of Surgical Research, Fort Sam Houston, TX, USA, \(^5\)Pfizer, Cambridge, MA, USA, \(^6\)Wake Forest School of Medicine, Winston-Salem, NC, USA.

Disclosures:

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Introduction: High energy craniofacial injuries offer a challenge for repair due to the complex architecture of the underlying bone in the area. The feasibility of using autograft including bone harvested from the patients costal, iliac, or calvaria bones, is often limited due to other injuries when severe facial reconstruction is required. Combination products of biomaterials and protein growth factors such as recombinant human bone morphogenetic protein 2 (rhBMP-2) are proving to be a successful clinical alternative to autografts in promoting bone regeneration. While collagen rhBMP-2 carriers (Medtronic’s Infuse\(^6\)) have shown robust regenerative capabilities, adverse side effects such as ectopic growth have limited their use to several specific conditions. Keratin (KTN) biomaterial carriers for rhBMP-2 offer potential advantages compared to collagen including controlled rates of degradation in vivo (due to lack of keratin-degrading enzymes), flexible material properties, and tunable rates of delivery. In this work, we compare the effectiveness of KTN as an rhBMP-2 carrier system to Infuse and autograft in a porcine mandible defect model.

Methods: Ten 40-50 kg Yorkshire pigs were given bilateral 3.5 cm x 1 cm osseous defects along the inferior border of the mandible with complete periosteal dissection using a model developed by Col Robert Hale and Dr. Pam Brown Baer at the US Army Institute for Surgical Research. The defect was generated via an extraoral surgical approach that included removal of the surrounding periostium. Given the extent of the defect, stainless steel fixation was required to insure mandible integrity remained throughout healing. One defect per pig was filled with autograft created from the bilateral bone removed during the creation of the defects. One group was treated with 3.5 mL of an injectable KTN biomaterial containing 0.1 mg/mL rhBMP-2 (0.35 mg per animal). The other group was treated with a small Infuse\(^6\) kit (Cat. # 7510200, Medtronic, Minneapolis, MN), a collagen-base carrier system, which delivers 1.5mg/mL rhBMP-2 (4.2 mg per animal). Micro-CT analysis was performed at 30 and 90 days post-treatment to assess defect volume and volume of ectopic bone growth using Osirix 3D Imaging Software. Mandibles were extracted after humanely euthanizing the animal at 90 days post-op, and were decalcified in Formical. Slices of the mandible were cut from within the center of the defect region, formalin fixed, paraffin embedded, cut to 5 μm sections, and placed on slides. Two sections per defect were stained with either Hemotoxylin and Eosin or Goldner’s Trichrome. For each slide, 4 random regions were imaged and scored on by a blindly observer on a scale of 1-4. In examining osteoid levels using Goldner’s Trichrome, the Mann-Whitney U-test was used to compare across treatment groups for both bone maturity and osteoid levels. Bone volume differences were determined by a paired T-test.

Results: Representative 3D rendering of Micro-CT images in the pig jaw at 30 days post-surgery are shown in Figure 1. KTN with rhBMP-2 treatment (indicated with the blue arrow) was able to fill the defect, while the autograft control (indicated with the red arrows) had not completely filled at this timepoint. At 30 days, the collagen-based carrier group (indicated by the white arrow) already contained nearly 3 times the bone volume of the KTN or autograft groups (Figure 2). At both 30 and 90 days, bone volume was not statistically different between the KTN with rhBMP-2 and autograft groups, but was significantly higher in defects with the collagen-base carrier at both time points (* P<0.005). Histological analysis of the pig jaws also confirmed that bone growth with KTN with rhBMP-2 is equivalent to that of the autograft control. Bone maturity scoring of the H&E section revealed that autograft and KTN with rhBMP-2 were not different at 1.95 and 2, respectively. The collagen-based carrier score of 2.5 was significantly different at the 2% level by the Mann-Whitney U-test. In examining osteoid levels using Goldner’s Trichrome, autograft and KTN with rhBMP-2 scored the same at 1.9, while the collagen-based carrier was again different between both groups, significantly different at the 2% level with a score 2.85. The Goldner’s Trichrome stain also showed that the KTN with rhBMP-2 had the highest amount of mineralized bone compared to autograft or the collagen-based carrier.

Discussion: Keratin biomaterials are a potential alternative to collagen-based carriers. The fact that mammals do not produce endogenous keratinase enzymes, allows for keratin materials to degrade hydrolytically and to remain stable in vivo. This degradation profile allows the KTN matrix to serve as a long lasting delivery vehicle and scaffold for cell infiltration in the regenerative microenvironment. We have previously shown that there is strong correlation between keratin degradation and drug release profiles (1, 2). This suggests that modulating the rate of keratin degradation can control rhBMP-2 delivery. The
injectable keratin-based carrier of rhBMP-2 achieved bone of equal quality to autograft and filled the defect faster. The keratin delivery system out performed the collagen-based carrier in terms of bone volume and quality though these results must be viewed cautiously given that this study used the clinical dose of rhBMP-2 in Infuse, a rhBMP-2 dosage that is much higher than is required for autograft equivalent regeneration in the KTN group. Given the challenges related to ectopic bone formation observed with collagen-based scaffolds, the healing profiles and reduction in ectopic bone formation observed with keratin-based carriers suggest the potential clinical utility of these materials in craniofacial bone regeneration.

**Significance:** Clinical collagen-based systems for delivery of rhBMP-2 such as Medtronic’s Infuse® have been associated with adverse side effects such as ectopic bone growth. Keratin biomaterials offer a naturally-based polymeric system that can provide tailored rhBMP-2 release profiles, reduce ectopic bone growth, and achieve comparable levels of bone healing to the standard of care, autograft, for mandible injuries.

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Figure 2: Bone volume calculations from the reconstructed micro-CT images. The KTN with rhBMP-2 performed equal autograft in its ability to close the defect. The collagen-based carrier was able to close the defect and showed exuberant bone growth generating nearly 3 times the volume as either KTN with rhBMP-2 or autograft (* indicates P<0.005).