Keratin Biomaterial for Delivery of rhBMP-2 Promotes Healing of Nonunion Bone Defect in Osteoporotic Model

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Introduction: Osteoporosis is a significant health problem for the elderly, resulting in fractures with high complication and mortality rates. Although the healing of a fracture in osteoporotic bone involves normal stages, the processes of mineralization and bone remodeling are prolonged (1,2). Recombinant bone morphogenic proteins (rhBMP) such as BMP-2 and BMP-7 have been shown in preclinical studies as potentially beneficial for stimulating the growth and regeneration of osteoporotic bone. However, there have been reports of ectopic bone formation as a complication after the use of collagen-based BMP-2 carrier (Infuse®, Medtronic Inc., Memphis, TN, USA) possibly due to an uncontrolled and rapid release of the growth factor (3).

Keratin biomaterial-based bone graft product (KeraGenics™ Bone, KeraNetics, Winston-Salem, NC, USA) is a biomaterial system based on keratin protein, which is extracted from human hair. Unlike other protein-based systems, humans do not produce keratinase enzymes that can degrade this material. It has been shown in previous studies that this carrier material can release various compounds such as small molecule drugs and growth factors (including BMP-2) in a manner parallel with the rate of the keratin hydrogels degradation (4). This ability to tune the levels and timing of growth factor release is considered a key advantage to a keratin system as it is known that sustained delivery of BMP-2 promotes more favorable new bone regeneration.

The primary objective of this study is to determine an efficacy of different formulations of keratin-based BMP-2 carriers for a repair of nonunion bone defect in a large animal model that has been conditioned for osteoporosis. The general hypothesis is that the keratin-BMP-2 biomaterials will be comparable to autograft, the current standard of care.

Methods: To test the hypotheses, osteoporotic sheep were created following the model developed by Lill et al.(5) Eleven 5-year-old sheep were ovariectomized, and received bi-weekly steroid injection, as well as being placed on a calcium/vitamin D-restricted diet for 7 months. The second surgery was performed to produce a 17 mm-diameter defect on each side of the iliac wings. One defect in all 11 animals was filled with morcellized autograft, representing a clinical standard to promote healing. The contralateral defect was filled with keratin material only (n=2), keratin injectable hydrogel with BMP-2 (n=6), or left empty (n=3). The empty defect served as a negative control, to also determine that the...
defect was maintained for the duration of the study. The injectable keratin with BMP-2 contained a total of 0.5 mg BMP-2.

The animals were euthanized 3 months after the second surgery. Tetracycline injections were given at 16 and 5 days before the euthanasia. The pelvic bones were harvested, trimmed, and contact radiographs were obtained. The percentage of bone within the defect was measured using the high-resolution contact radiographic images. The amount of bone regeneration was described as percentage of bone presented in the total area of the original defect. The data of each treatment was then statistically analyzed using t-test. All specimens were embedded in poly-methyl-methacrylate (PMMA) as described in a standard technique. The specimens will be sectioned, ground, and polished to optical finish. Backscattered electron (BSE) imaging with gray scale levels will be used to demonstrate the spectrum of bone remodeling and structural connectivity. The presences of double labeling in remodeling bone segments will be used to calculate mineral apposition rate (MAR) and demonstrate tissue viability.

**Results:** At the three-month end point, contact radiograph showed the empty defect had minimal new bone formation (34 ± 14%), demonstrating that the defect remained unhealed throughout the duration of the study and was indeed a nonunion defect. The keratin-only group trended towards a larger bone volume relatively to empty defect, but without statistical significance (70 ± 18%, p=0.09). (Figure 1) The autograft-filled defects had 90 ± 13% new bone formation, while the defects filled with injectable keratin with BMP-2 had an average bone growth of 98 ± 3%. The injectable keratin with BMP-2 group achieved a comparable result with the autograft-filled group (p=0.12). None of the treatments resulted in ectopic bone formation. Analysis of structural connectivity and bone remodeling will be performed under BSE, and the results will be disclosed at the time of presentation, as well as the histology and MAR.

**Discussion:** In comparison to the autograph representing the standard-of-care, injectable keratin-based BMP-2 carrier achieved a similar result in facilitating bone healing. We did not find heterotopic ossification in any specimen treated with keratin material. Case reports of ectopic bone growth after the use of BMP-2 in spinal fusion surgeries suggested the phenomenon is dosage-dependent (8, 9). The unique characteristic of the keratin system, which gradually releases a sustained, small amount of BMP-2 can potentially decrease the incidence of ectopic bone formation, but extended studies are required.

**Significance:** Keratin biomaterial-based bone grafts had similar efficacy in promoting bone regeneration comparing to autograft. This finding indicates that the tested material has a potential for the use as bone substitute in a circumstance where the autograft is limited. The technique similar to the protocol used in this study can be applied to create a non-union pelvic bone defect in osteoporotic sheep after the 12-week study period.
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Contact Radiography

Empty | Autograft | Injectable Keratin | Injectable Keratin + BMP

A | B | C | D

% Bone

A | B | C | D

33.7% | 89.5% | 70.0% | 98.2%