Controlled BMP2 Release from Keratin-based Hydrogels Modulates Osteoinduction

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Statement of Purpose: Tunable degradation of polymers is important for tissue engineering to control the release of biologicals and allow vascular and cellular ingrowth. Keratin isolated from human hair can be oxidatively-extracted (keratose) or reductively-extracted (kerateine) to alter crosslinking. Keratose is less crosslinked than kerateine and degrades more rapidly. Keratin-based biomaterials have wide applications in tissue engineering, particularly as drug-release materials. Clinically, bone morphogenetic protein-2 (BMP2) is delivered with a collagen sponge to induce bone. However, the uncontrolled release of BMP2 can cause ectopic bone or severe inflammation. Our group has demonstrated that keratose-based materials could be tailored to deliver BMP2 in a manner that followed their rate of degradation. The aim of the current study was to examine the ability of keratin-derived materials containing BMP2 to induce bone formation in vivo.

Methods: Osteoinduction of keratose gel, kerateine gel, and keratose:kerateine blends (30:70, 50:50, 70:30) ± 0.67µg BMP2/mg material was examined in comparison to collagen sponge in a well-characterized mouse model. Samples (25µl gel; 1cm^2 collagen sponge) were/ placed in gelatin capsules and implanted bilaterally in pockets created in the gastrocnemius of 6-8-week-old male athymic nude mice (n=8 implants/variable). After five weeks, new bone formation was assessed qualitatively by histology and quantitatively by microCT and histomorphometry. Data are mean±SEM and were analyzed by ANOVA with posthoc Bonferroni's modification of Student's t-test.

Results: All samples containing BMP2 induced new bone formation on microCT (Figure 1A) and histology, while no new bone was seen in samples without BMP2 (Figure 1B). On microCT, the most bone formation was seen in 50:50+BMP2. 30:70+BMP2 had significantly more new bone volume than collagen+BMP2 or keratose+BMP2. Histologically, bone formation amount and character were altered by keratin materials. 30:70+BMP2 had significantly higher total bone area than collagen+BMP2 and keratose+BMP2. 70:30+BMP2 had significantly more cortical-type bone and bone marrow than keratose+BMP2. However, 30:70+BMP2 had significantly more material still present than collagen+BMP2, keratose+BMP2, or 50:50+BMP2. Keratose (±BMP2) degraded completely over the five weeks. In samples without BMP2, the material remaining increased with increasing kerateine.

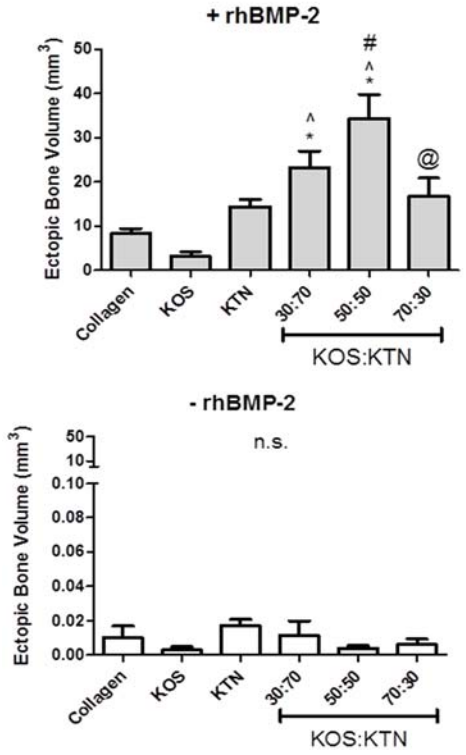

Figure 1. MicroCT analysis of new bone volume formed in muscle pouch model of samples with (A) or without (B) rhBMP2. *p<0.05 vs. Collagen Sponge; ^p<0.05 vs. KOS; #p<0.05 vs. KTN; @p<0.05 vs.30:70 $p<0.05 vs. 50:50 ~p<0.05 vs. 70:30.

Conclusion: Keratose/kerateine carriers facilitate greater BMP2-induced osteoinduction than the standard collagen or either biomaterial separately. These carriers induced new bone with varying histological appearances, an effect which needs to be studied. Our results suggest that keratin-based materials are suitable as slow-release BMP2 carriers for clinical bone regeneration.