

Delivery of BMP-2 from Keratin Hydrogels with Tunable Rates of Degradation

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Statement of Purpose: Delivery of bone morphogenetic protein 2 (BMP-2) from resorbable collagen sponges is an example of the successful clinical application of an off-the-shelf biomaterial for bone regeneration. However, challenges that have limited the application of these materials include a rapid degradation of the collagen material, burst release of BMP-2 from the collagen sponges, and subsequent ectopic bone formation. We have been exploring keratin biomaterials as an alternative carrier system for BMP-2. Keratins are intermediate filament proteins that can be derived from various sources including human hair. Humans are not known to express keratinase enzymes and therefore these materials degrade relatively slowly in vivo, providing a mechanism to slow the rate of degradation of the scaffold (osteoconductive matrix) on which bone regeneration occurs. In addition, keratins can be extracted from hair either oxidatively (yielding a form of keratin known as keratose; KOS) or reductively (yielding a form of keratin known as keratine; KTN). KOS does not contain disulfide crosslinks and degrades more rapidly while KTN does contain disulfide crosslinks and degrades more slowly. Thus, the rate of the keratin carrier degradation can also be controlled through a process of mixing KOS and KTN, providing materials that can be tuned to the timeframe required for regeneration of a specific injury.

Methods: Oxidatively (KOS) or reductively (KTN) extracted keratin was obtained from KeraNetics, LLC as a lyophilized powder [1]. KOS or KTN hydrogels were formed at 15% w/v by adding water or water containing BMP-2 and allowing to gel overnight at 37°C. Mixtures of KOS-KTN were prepared in the same fashion (15% w/v) by mixing the lyophilized powders at the desired ratios (70:30, 50:50, or 30:70 for KOS:KTN, respectively) before addition of water. In some experiments, BMP-2 was labeled with AlexaFluor555 or DyLight800 to allow fluorescent detection of BMP-2 in vitro or in vivo, respectively. For in vitro degradation and release experiments, keratin hydrogels of known volumes were placed into 1.5 mL conical tubes and then layered with PBS. At specified times, the PBS was removed. Keratin content was determined by a Lowry protein assay while BMP-2 release was determined by fluorescence at 480/555nm on a Biotek platereader. For in vivo experiments, a 8mm critically-sized defects were created in the left legs of a male Sprague-Dawley rats (~375 grams). Keratin hydrogels (KOS, KTN, or KOS:KTN mixtures) containing BMP-2 labeled with DyLight800 (near-IR fluorophore) were implanted at the defect site and were imaged either immediately after surgery at one-week post-surgery. The amount of BMP-2 remaining at the implant site was then determined.

Results: Figure 1 shows the rate of keratin degradation in vitro as determined by DC protein assay. The rate of degradation was tuned by varying the ratio of KOS:KTN with those formulations containing more KOS degrading more rapidly. Figure 2 shows BMP-2 release (as determined by AF555 fluorescence) for the various keratin formulations. No difference was observed in the rates of release from the various keratin hydrogel formulations in vitro. However, in vivo, the effects of the hydrogel degradation rate appear to have implications for the retention of BMP-2 at a bone defect site. Figure 3 shows results of BMP-2 retention in a critically-sized rat femur defect model. (A) shows X-ray of defect at time of surgery and (B) shows the amount of BMP2 fluorescence immediately after surgery; the two locations of high intensity signal in (B) are from muscle cut away from implant site and BMP-2 that had leached into that muscle tissue. This can be compared to the amount of BMP-2 remaining at the implant site when delivered from KTN (C) or collagen (D) as determined by DyLight800 signal intensity on a Bruker imaging system. Most of the BMP-2 was gone from the site of implant at 1 week post-op, but ~ 5% of the BMP-2 remained when implanted with KTN compared to none (detectable) with collagen.

Figure 1. Degradation of keratin hydrogels in vitro.

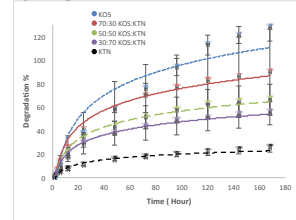


Figure 2. BMP-2 release from keratin hydrogels in vivo.

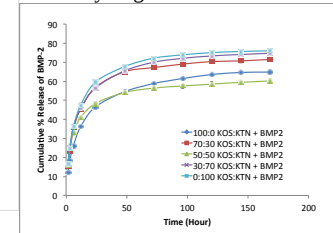
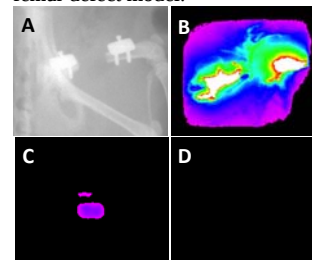


Figure 3. BMP-2 persistence at implant site in critically-sized femur defect model.



Conclusions: Keratin hydrogels can be tuned for degradation simply by mixing the forms obtained by oxidative or reductive extraction (KOS and KTN, respectively). This tunability has implications for the temporal control of BMP-2 delivery in vivo and may provide a means to tune material degradation

properties to match timeframes required for healing of different bone injuries. We are currently assessing the timelines of BMP-2 release from various keratin formulations and from collagen in the rat femur defect model in vivo.

References: Kowalczewski CJ et al. Bioamaterials 2014; 35: 3220-3228.