Keratose Biomaterial as a Carrier Matrix of BMP2 and Stem Cells for Bone Regeneration

R. C. de Guzman, T. Teli, S. L. Ray, M. D. Ellenburg, J. M. Saul, T. L. Smith, and M. E. Van Dyke
Wake Forest Institute for Regenerative Medicine, Winston-Salem, NC

Statement of Purpose: Repair of critical-size load-bearing bone defects is still a major clinical challenge. To date, the ability to completely form a remodeled tissue with organizational resemblance to normal bone while restoring its full biomechanical properties has not been demonstrated. Multiple third-generation ceramic and polymeric biomaterials are currently being explored to drive the host body to repair itself and synthesize a native bone. Hard keratins may be considered for this application since they are biocompatible and degradable in a controlled manner without producing any toxic byproducts. Moreover, they form gels which can be employed as a carrier matrix. Specifically in this study, oxidized keratins called keratoses were investigated for their potential to deliver a potent osteoinductive factor, bone morphogenetic protein 2 (BMP2), and to support adipose tissue-derived mesenchymal stem cells (ADSCs) that can differentiate into mature osteoblasts for bone regeneration.

Methods: Keratose fractions (crude, alpha, and gamma) were extracted from human hair based on isoelectric and chromatographic techniques, sterilized via ionizing radiation, and reconstituted as 0.02% coating solutions or as 20% gels. Polystyrene culture plates were then keratose-coated, seeded with passage-2 ADSCs that were isolated from subcutaneous fat following lipoplasty, and cells were maintained in bone differentiation media. Quantitative PCR was performed using primers for osteoblastic activity: runt-related transcription factor 2 (RUNX2), collagen type I alpha 1 (COL1A1), alkaline phosphatase liver/bone/kidney (ALPL), and bone gamma-carboxyglutamate (BGLAP) (coding for the osteocalcin protein). ALPL expression was verified via colorimetric assays. Keratose gels were loaded with BMP2 and the release curves of proteins were determined *in vitro* using Lowry and ELISA. The bone-forming capacity of keratose-BMP2 was tested by injecting the gels in mouse femoral muscles and micro-computed tomography (μ-CT) analysis after 2 weeks. A bone regeneration experiment was conducted in a rat critical-size defect model by removing an 8-mm segment of the femur and placing the keratose-BMP2 gel between the bone stumps within a collagen I tube. Rats were allowed to recover and the progression of bone formation was monitored through x-ray fluoroscopy and μ-CT. Regenerate tissues were collected and processed by histological staining.

Results: After 3 weeks in culture, differentiated ADSCs on keratose coatings produced higher ALPL transcripts compared to cells on control uncoated surface. Crude and gamma fractions induced ~3-fold increase of BGLAP (Fig. 1), indicative of enhanced osteoblast maturation. Crude keratose gels mixed with BMP2 demonstrated overlapping release profiles (Fig. 2A) suggesting that BMP2 binds to keratose and its delivery can be regulated by controlling the keratose degradation rate. BMP2, held by keratose gel, was found to be bioactive as evidenced by trabecular bone formation within mouse leg muscles (Fig. 2B). Preliminary keratose-200 μg BMP2 implantation test in a critical-size rat femur defect showed faster and more neo-bone regeneration (Fig. 3) than in rats with collagen I tube alone. Masson's trichrome staining revealed residual keratins in the regenerate as predicted by the release trendline. Immunohistochemistry detected the presence of active osteoblasts in the newly-formed bone tissue.

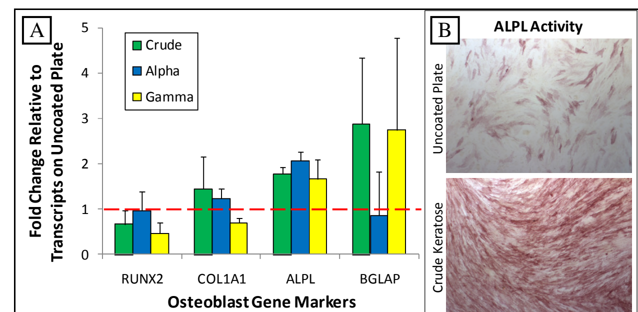

Figure 1. A) Gene transcription in ADSCs cultured on keratose-coated plates. B) Differentiated osteoblasts express ALPL.

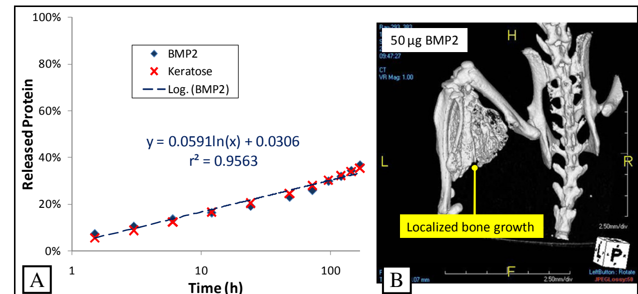

Figure 2. A) Release curves of keratose and BMP2. B) Keratose-BMP2 gel induced localized ectopic bone formation.

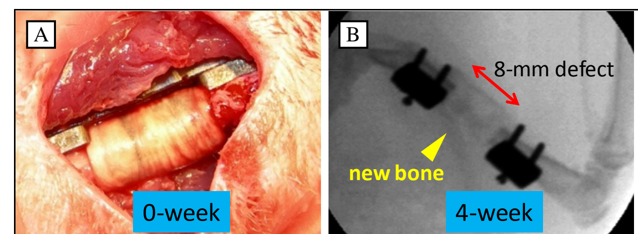

Figure 3. A) Keratose-BMP2 gel held by a collagen I tube in the femur defect model. B) Mineralization of bone gap after 4 weeks.

Conclusions: Keratose biomaterial was exhibited to support the differentiation of ADSCs to functional osteoblasts, suggesting that ADSCs in keratose gel may be a feasible tissue-engineered construct. Keratose was also shown to be an effective BMP2-delivery system for regeneration of critical-size bone defects. Additionally, the combination of BMP-2 and stem cells in a keratose matrix may provide a potent mechanism for bone repair.