Progenitor Cell-Free and Exogenous Growth Factor-Free *In Vivo* Bone Regeneration Using Nanoparticulate Mineralized Collagen Scaffolds

Xiaoyan Ren MD, PhD, David Bischoff PhD, Daniel W. Weisgerber BA, Dean T. Yamaguchi MD, PhD, Timothy A. Miller MD, Brendan A.C. Harley ScD, Justine C. Lee MD, PhD
UCLA Division of Plastic and Reconstructive Surgery, Los Angeles, CA

Background: Current strategies for skeletal regeneration often require co-delivery of scaffold technologies, growth factors, and cellular material. However, isolation and expansion of stem cells can be time consuming, costly, and requires an additional procedure for harvest. Further, the introduction of supraphysiologic doses of growth factors may result in untoward clinical side effects, warranting pursuit of alternative methods for stimulating osteogenesis.
Methods: BMSCs harvested from long bones were cultured in osteogenic media on non-mineralized (Col-GAG) and nanoparticulate mineralized (MC-GAG) collagen glycosaminoglycan scaffolds. Scaffolds were implanted in 14 mm cranial defects for 12 weeks, explanted, and analyzed. Gene and protein expression were measured using quantitative real time RT-PCR and western blot analysis. Scaffolds were subjected to histochemical and micro-computed tomographic analyses for mineralization.
Results: In vitro cultures of BMSCs on Col-GAG and MC-GAG demonstrated elevated osteogenic gene expression and mineralization on MC-GAG scaffolds. In addition, elevated expression of differential BMPs were found on MC-GAG but not Col-GAG scaffolds. Western blot analysis demonstrated constitutive activation of a downstream BMP receptor signalling molecule, Smad1/5, in MC-GAG scaffolds. Cranial defects with implanted MC-GAG scaffolds demonstrated mineralization at 50-80% the density, biomechanical strength, and stiffness as native calvarial bone, whereas Col-GAG scaffolds were similar to unreconstructed cranial defects. There were no statistically significant differences between empty MC-GAG, MC-GAG treated with BMSCs, or MC-GAG treated with BMSCs and BMP-2.
Conclusions: Nanoparticulate mineralized collagen glycosaminoglycan scaffolds demonstrate *in vivo* bone healing in the absence of *ex vivo* expanded osteoprogenitors or exogenous growth factor stimulation.