Tissue Response to Hydrogel Scaffolds Delivering Bone Morphogenetic Protein-2 for Bone Augmentation

L.A. Kinard[1], R.L. Dahlin[2], A.M. Henslee[2], P.P. Spicer[2], C. Chu[2], F.K. Kasper[2], A.G. Mikos[1,2]
[1]Department of Chemical and Biomolecular Engineering, [2]Department of Bioengineering, Rice University, Houston, TX

Statement of Purpose: Injectable biomaterials are favored to regenerate craniofacial bone due to their noninvasive application and propensity for contouring to the existing bone surface, which is of unique interest to the treatment of facial bone deformities. An injectable hydrogel system composed of oligo(poly(ethylene glycol) fumarate) (OPF) and gelatin microparticles (GMs) has been developed and tested in our laboratory for tissue engineering of bone and cartilage.[1] In the present work, OPF-GM composites delivering bone morphogenetic protein-2 (BMP-2), an osteoinductive agent, were implanted at an orthotopic site in rats to assess the tissue response and extent of vertical bone augmentation.

Methods: OPF synthesis and GM fabrication followed established methods.[2,3] OPF was synthesized from 3.4 kDa PEG (Sigma, St. Louis, MO), and the product was characterized using GPC and ^{1}H NMR. GMs were fabricated using acidic gelatin (Nitta Gelatin Co., Osaka, Japan), cross-linked with 10 mM glutaraldehyde, and sieved to 50-100 μm. Composite scaffolds were fabricated by incorporating pre-swollen GMs (0.44 g GM/g OPF; dry basis) into the OPF precursor solution. The experimental groups were loaded with 40 ng BMP-2/ml scaffold (Peprotech, Rocky Hill, NJ) in either the GM phase (BG) or the bulk OPF phase (BO). A composite scaffold without BMP-2 and a surgery where no scaffold was implanted served as the material control (MC) and empty control (E), respectively. A vertical bone augmentation model was developed for this experiment in which two scaffolds were placed directly above the left and right parietal bone of the skull of 12 week old, male, Fischer 344 rats (Harlan Laboratories, Inc., Indianapolis, IN). Scaffolds in each animal were contained within separate polypropylene cassettes fastened to the skull with screws so that the scaffolds were exposed only to the bone surface and enclosed on the top and sides by the cassette. A sagittal incision was made over the scalp from the nasal bone to the middle sagittal crest; the periosteum was resected; the surface of the parietal bone was roughened with a rounded bur; pilot holes were drilled; 0-80 stainless steel screws (Small Parts, Inc., Logansport, IN) were inserted to secure the cassettes; and the scalp was closed. Animals were euthanized 28 days post implantation, and samples were evaluated by μCT and histology.

Results: A quantitative analysis of augmented bone was carried out using μCT, and the difference in newly formed mineralized bone was insignificant across all groups as shown in **Figure 2**. Surprisingly, dissolution of minerals was apparent in a number of samples indicated by a decrease in radiopacity, and this outcome was correlated with the occurrence of bone resorption by histology. Although the level of resorption varied widely between samples, bone resorption occurred in samples across all groups. Since there are several potential explanations for the bone resorption that occurred, further investigation would be needed to determine the definitive cause. To further investigate the tissue response, histology was performed on representative samples. In general, dynamic bone remodeling was observed in the immediate vicinity of all scaffolds analyzed by histology. Newly formed mineralized bone and the presence of osteoid were rarely observed superior to the original bone surface; one such example is shown in **Figure 1** where the deep blue staining labeled NB indicates mineralized bone that formed within the scaffold volume during the implantation period. Formation of osteoid, which stains red, is also apparent in **Figure 1** adjacent to the new bone.

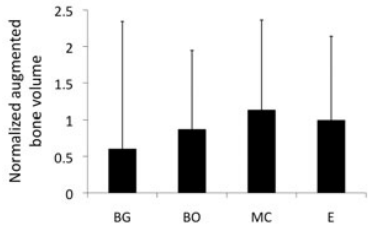

Figure 2: Volume of augmented bone normalized to the empty control.

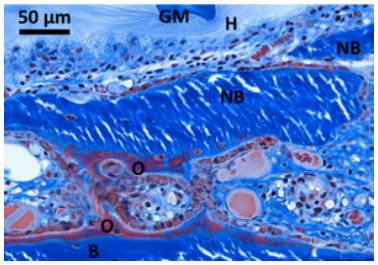

Figure 1: Goldner's trichrome stained section (20X). NB=New bone, B=Bone, O=Osteoid, H=Hydrogel, GM=Gelatin Microparticle

Conclusions: For OPF-GM composites and empty controls, no difference in augmented bone volume was observed between any of the groups. Bone resorption occurred in samples across all groups, but the cause remains to be elucidated. Augmented bone was rarely observed, but evidence of augmentation in some samples was supported by μCT and histological findings. Further investigation of the current animal model is warranted to determine the mechanism underlying the bone resorption observed. Additionally, the inclusion of a longer duration of implantation in future studies will enable investigation of the long-term effects of the implanted scaffolds. The results collectively suggest that the OPF-GM composite hydrogel system delivering BMP-2 should be further studied in order to fully determine and optimize the potential of this scaffold as an injectable biomaterial for craniofacial bone augmentation.

Acknowledgements: The work described in this abstract was supported by a grant from the Armed Forces Institute of Regenerative Medicine (W81XWH-08-2-0032). L.A.K. acknowledges support from an NSF Graduate Fellowship (0940902).

References: 1 Guo X. Acta Biomaterialia. 2010;6:39-47. 2 Kinard LA. Nat Protoc. 2012;7:1219-1227. 3 Tabata Y. Journal of Biomaterials Science-Polymer Edition. 1999;10:79-94.