GFOGER Peptide-Modified Matrices Support Osteogenic Differentiation and Bone Formation

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Statement of Purpose: Non-union defects account for 10-15% of the 6 million fractures which occur each year. Although autografts and allografts are the standard treatments for non-union fractures, they are limited by lack of tissue availability, pain and possible disease transmission. Therefore, there is a need for improved bone graft technologies. While much effort has been focused on using the RGD adhesion motif to functionalize bone materials, this approach has shown disappointing results [1], possibly due to the absence of modulatory domains or other features found in native ECM ligands and lack of specificity to pro-osteogenic integrins. To address this limitation of RGD, we have developed a synthetic GFOGER peptide which mimics the native tertiary structure of collagen and binds specifically to the pro-osteogenic alpha2beta1 integrin [2]. Here we investigate the effect of a GFOGER-modified polyethylene glycol (PEG) hydrogel on the osteogenic differentiation and on bone formation. **Methods:** In vitro, 2mM GFOGER-PEG and RGD-PEG gels were cast in 6-well plates to create peptide-modified surfaces. Human mesenchymal stem cells (hMSCs) were cultured in osteogenic media on the GFOGER and RGD surfaces and assayed for viability by Live/Dead staining, as well as for alkaline phosphatase (ALP) activity at 10 and 9 days post-induction respectively. In vivo, 2.5mm defects were created in the right radii of 6-11-week old mice, leaving the ulna intact. 2mM GFOGER-PEG or PEG-only hydrogels were cast in polyimide tubes, and press-fit into the defects. Following implantation, bone formation was examined using Faxitron X-ray imaging and micro computed tomography (μCT). **Results:** hMSCs maintained viability at 10 days post-induction on both RGD and GFOGER hydrogel surfaces. Cells did not adhere to unfunctionalized PEG. However, ALP activity in hMSCs was significantly higher on GFOGER than on RGD 9 days after osteogenic induction. In vivo, defects treated with GFOGER showed an increase in bone volume and bone mass at 8 weeks post-implantation.

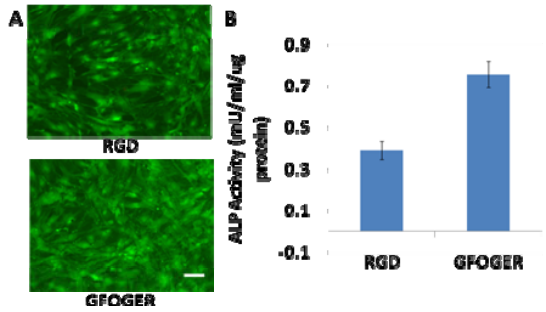

Figure 1. (A) Live/Dead staining of hMSCs on RGD (top) and GFOGER (bottom) hydrogels at Day 10. p=0.016, with Student's t-test. (B) Alkaline phosphatase activity of hMSCs on RGD, GFOGER at Day 9 (n=4). Scale bar 20μm.

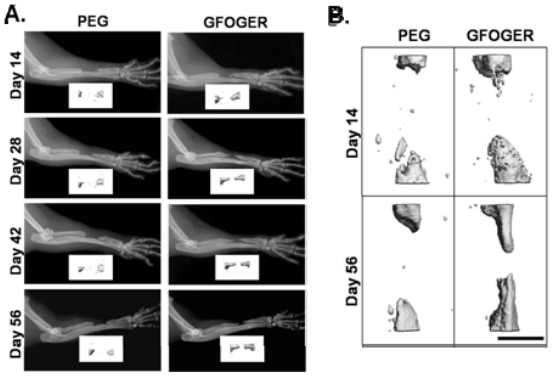

Figure 2. (A) Faxitron images of mouse radii and ulnae at 14, 28, 42 and 56 days post-surgery with insets of corresponding μCT reconstructions of the radial defects. (B) μCT reconstructions of bone formation within defects at 14 and 56 days. Scale bar at 1.0mm.

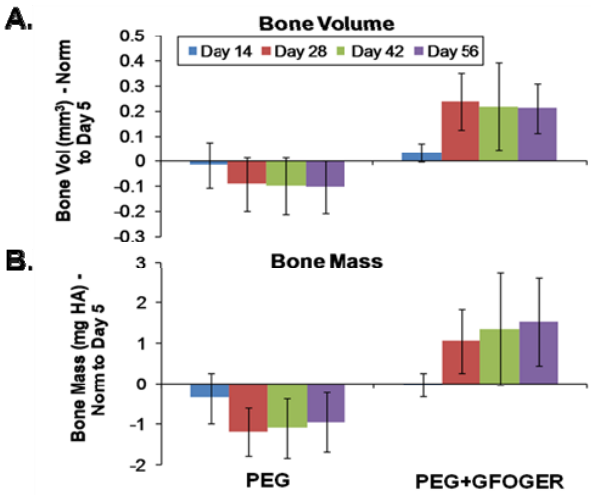

Figure 3. (A) Bone volume and (B) mass within mouse radial defects implanted with PEG or PEG+GFOGER hydrogels by MicroCT analysis at 14, 28, 42 and 56 days post-surgery (n=3). ANOVA of bone volume and mass using time and treatment as categorical variables gave p values of <0.005 and 0.006 respectively for treatment effects.

Conclusions: Our data demonstrates that GFOGER enhances osteogenic differentiation of human stem cells in vitro compared to RGD. Preliminary in vivo data also suggests that GFOGER-modified PEG hydrogels also support bone formation in vivo within a critical-sized mouse radial defect.

References: [1] Barber TA, et al., *J Biomed Mater Res A*, 80:306-320 (2007); [2] Morton LF, et al., *Biochem J,* 299:791-797 (2004);