VEGF-Functionalized Integrin-Specific Hydrogels for Increased Stem Cell Proliferation, Vascularization and Regeneration of Critically-sized Bone Defects

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Statement of Purpose: Non-healing bone defects and fractures represent a serious clinical problem with over 600,000 bone replacement procedures performed each year. Bone tissue engineering offers an alternative to traditional auto- and allograft techniques; however, many tissue engineered constructs fail to show significant bone regeneration. The lack of an appropriate vascular network in tissue engineered scaffolds has emerged as a major limitation that prevents full regeneration of bone especially when constructs contain stem cells[1]. We have shown that hydrogels functionalized with the collagen-mimetic peptide GFOGER enhance bone regeneration compared to RGD-presenting hydrogels[2]. However, without vasculogenic cues, delivery of stem cells within scaffolds often results in significant cell death and impaired healing. The objective of this project is to investigate the effect of differing ligands and associated integrin activation along with the incorporation of vascular endothelial growth factor (VEGF) for increased survival of stem cells and associated bone regeneration in a critically-sized defect.

Methods: PEG-MAL (20 kDa, Laysan Bio) macromer was reacted with $VEGF_{165}$ along with either the adhesive peptide $GGYGGGPG(GPP)_5GFOGER(GPP)_5GPC$ (GFOGER) or CRGDSPC (RGD). Macromers were cross-linked with MMP-cleavable peptide GCRDVPMSMRGGDRCG. Covalent tethering of VEGF was verified via SDS-PAGE gel.

Segmental bone defect: A non-healing (2.5 mm) segmental defect was created in the radii of mice, followed by implantation of hydrogels with differing ligands and VEGF doses. Bone regeneration was monitored through microcomputed tomography (μCT) at 4 and 8 weeks. Vascularization of defect regions were assessed by perfusion of a radiopaque contrast agent and μCT. For hMSC implantation, luciferase-expressing hMSCs were encapsulated within hydrogels and imaged using bioluminescence. The study involving luciferase-expressing hMSCs is in progress.

Results: To assess the effect of VEGF on bone formation, hydrogels presenting either RGD or GFOGER adhesive peptides as well as differing doses of VEGF were implanted into critically-sized radial bone defects in mice. At 4 weeks, there was an increased amount of regenerated bone volume for scaffolds containing 50 ng VEGF compared to no VEGF for both adhesive peptides. GFOGER-presenting hydrogels had higher amounts of bone regeneration compared to RGD-presenting hydrogels at 0 and 250 ng doses of VEGF (Fig. 1A). In addition to heightened bone regeneration, VEGF doses as low as 30 ng within GFOGER-presenting hydrogels resulted in marked increases in vascularization within the defect compared to empty defects and hydrogels without VEGF at 8 weeks (Fig. 1B). With these effects in cell-free scaffolds, the effect of VEGF incorporation within stem cell-encapsulated hydrogels in the bone defect was assessed. When implanted into the critically-sized radial bone defects, GFOGER-presenting VEGF-functionalized hydrogels with luciferase-expressing human mesenchymal stem cells (hMSCs) exhibited higher bioluminescent signal at day 7 compared to hydrogels without VEGF. This increase in bioluminescent signal with increasing VEGF dose is not seen in RGD-presenting hydrogels (Fig. 1C,D).

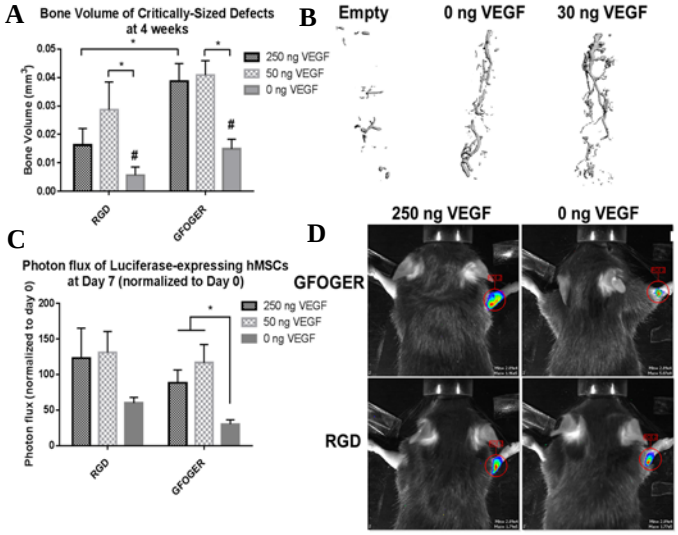

Figure 1: A) *Quantification of bone volume at 4 weeks via μCT. # indicates p<0.05 RGD vs. GFOGER at 0 ng VEGF dose.* **B)** *Images of Microfill vascular perfusion showing heightened vascularization at 30 ng VEGF compared to empty defects and 0 ng VEGF.* **C)** *Bioluminescent signal in critically-sized bone defects at day 7.* **D)** *Representative images of bioluminescent quantification. Statistics: * represents p<0.05*

Conclusions: We demonstrate the ability for VEGF to increase the bone regenerative capacity as well as the vasculogenic capability of hydrogels in a critically-sized radial segmental bone defect. We also show increased stem cell numbers within hydrogels with VEGF at day 7 after implantation. Interestingly, this increase in hMSC signal in the presence of VEGF was solely limited to hydrogels presenting the $\alpha_2\beta_1$ specific GFOGER sequence suggesting synergy between VEGF and this integrin. The results of the on-going hMSC-encapsulated hydrogels within the defect will determine whether the increase in hMSCs within scaffolds containing VEGF will translate to heightened bone regeneration. Future studies will be performed investigating the effect of activating the $\alpha_2\beta_1$ integrin on VEGF receptors along with downstream signaling pathways.

References 1. Santos M. et al. Macromol. Bio. 2009 10: 12-27. 2. Shekaran A. et al. Biomaterials 2014 35:5453-61.

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