PEG Hydrogels Functionalized with a Collagen mimetic Peptide and Vascular Endothelial Growth Factor Promote Enhanced Network Formation of HUVECs and Therapeutic Value in Bone Defects

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Introduction: Non-healing bone defects and fractures represent a serious clinical problem with over 600,000 bone replacement procedures performed each year in the U.S. and costing over 5 billion dollars annually. Bone tissue engineering offers an alternative to traditional auto- and allograft techniques which are limited by tissue supply, morbidity, poor bioactivity, and the possibility of disease transmission. 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 one of the main limitations that prevent full regeneration of endogenous bone[1]. Our group has previously shown that coating of a scaffold with the collagen-mimetic peptide GFOGER enhances bone regeneration and bridging of a critical-sized defect when incorporated onto a PCL scaffold[2]. The purpose of this study is to investigate the effects of presenting GFOGER and vascular endothelial growth factor (VEGF) in a hydrogel vehicle to enhance vascularization in a murine segmental bone defect model. We hypothesize that the incorporation of VEGF into our constructs will improve the therapeutic value of GFOGER scaffolds.

Materials and Methods:

GFOGER/VEGF hydrogel preparation and release characterization: PEG-MAL (20 kDa MW, Laysan Bio) precursor was reacted with rhVEGF$_{165}$ (Invitrogen) GGYGGGPG(GPP)$_5$GFOGER(GPP)$_5$GPC (GFOGER). Functionalized macromers were cross-linked into a hydrogel via GCRDVPMSMRGGDRCG (AAPPTEC). To study release, VEGF was tagged with Alexa Fluor 488. PEG-GFOGER-VEGF hydrogels were then placed in collagenase I solution or PBS and samples subsequently analyzed for fluorescence.

Endothelial network formation on surface of hydrogels: Human umbilical vein endothelial cells (HUVECs, Lonza) were seeded onto hydrogels with either PEG-GFOGER-VEGF or PEG-GFOGER. Cell samples were incubated for 4, 8 and 15 hr at which cells were fixed, permeabilized and stained with DAPI and phalloidin followed by analysis through ImageJ.

Segmental bone defects: NOD.CB17-Prkdc^scid mice underwent surgery to remove a
critically-sized segment of radial bone followed by implantation of PEG-GFOGER hydrogels with 0, 1, 10, or 30 ug/mL VEGF or left empty. This study is still in progress. Bone regeneration is monitored through micro-computed tomography at 4 and 8 weeks.

Results: ***VEGF Engraftment and Release:*** Upon exposure to collagenase, 100% of the VEGF was released from gels as opposed to gels incubated in PBS that retained approximately 41% and 62% of the incorporated VEGF in 4.0 and 7.5%wt gels respectively (Figure 1A).

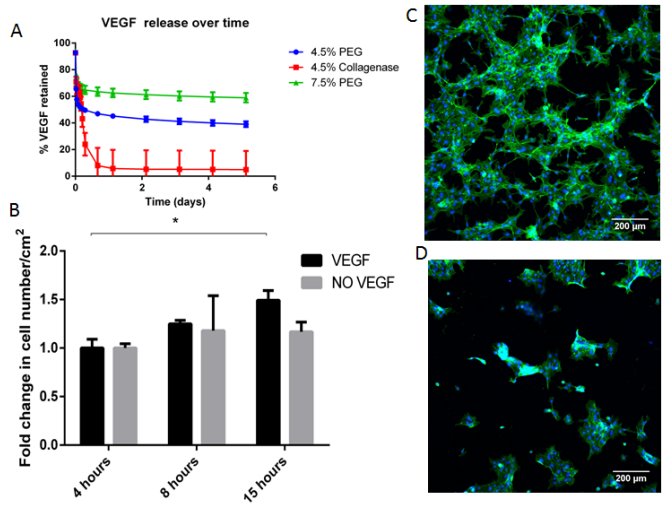

Figure 1: **A)** VEGF release profile **B)** Cell quantification on 2D gels. Representative images of cells on 2D hydrogels at 15 hours for hydrogels **C)** with VEGF or **D)** lacking VEGF.

Angiogenic potential of HUVECs in functionalized PEG hydrogels: To assess biological activity, HUVECs were seeded on top of hydrogels containing engrafted VEGF or no VEGF. After 15 hours, cells on hydrogels with engrafted VEGF show significantly higher cell numbers and enhanced network formation as compared to cells seeded on hydrogels lacking VEGF (Figure 1B-D).

Conclusions: The current work demonstrates the ability to incorporate a vascular growth factor along with a collagen-mimetic peptide in an effort to improve the regeneration of segmental bone defects. Immobilization of VEGF onto the PEG backbone significantly enhances the biological functionality of the scaffold and is a more effective method of delivering growth factor due to the localized profile. Incorporation of a collagenase-sensitive cross-linker (VPM) also allows for covalently bound VEGF to be released as cells invade thus increasing its effectiveness on vascularization in vivo. Further studies will determine the extent of vascularization through perfusion microCT methods.

References:

1. Santos M. et al. Macromol. Bio.2009 10(1) 12-27
2. Wojtowicz et al. Biomaterials 2010 31 2754-2582

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