Covalently Immobilized PDGF-BB Stimulates Angiogenesis in Poly (ethylene glycol) Based Hydrogels

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Statement of Purpose: The field of tissue engineering is severely limited by tissue engineered constructs' lack of microvascularization, which is necessary for transport of nutrients, oxygen, and waste. Platelet-derived growth factor BB (PDGF) is a key angiogenic protein able to support neovessel stabilization by inducing functional anastomoses and recruiting pericytes (1). Due to PDGF's widespread effects in the body and half life of only thirty minutes in circulating blood, local delivery and covalently immobilized PDGF may be necessary. Covalently immobilized PDGF was synthesized, and its bioactivity was confimed. PEGylated PDGF was then shown alone and in combination with PEGylated FGF in PEG-based hydrogels to induce HUVEC migration and tubulogenesis in 2D, 3D, and *in vivo* in the mouse cornea micropocket model (2).

Methods: PEG (MW = 6 kDa; Fluka, Milwaukee, WI) was acrylated by reaction with acryloyl chloride (3). PEG-succinimidyl carbonate-monoacrylate was reacted with RGDS in sodium bicarbonate buffer at a pH of 8.5 to form PEGylated RGDS. PEGylated basic fibroblast growth factor (FGF), PDGF, and vascular endothelial growth factor (VEGF) were made in a similar manner. 2D studies were performed by modifying the surface of a bulk PEGDA hydrogel as previously described (4) with a solution of 50 mg./mL PEG-RGDS, 17.6 µg/mL PEG-PDGF and/or 4.4 µg/mL PEG-FGF. HUVECs and/or 10T1/2 cells were seeded onto the modified gels and imaged using a fluorescent microscope. Cell-tracker labeled HUVECs were encapsulated into degradable hydrogels containing an MMP-sensitive peptide in the polymer backbone with 2µg/L PEG-PDGF and/or 0.5µg/L PEG-FGF and imaged over time on a confocal microscope. *In vivo* studies were performed by implanting a degradable hydrogel containing 320 ng VEGF per gel and 3.2 ng PEG-VEGF, or 320 ng PDGF, 3.2 ng PEG-PDGF, 80 ng FGF, and 0.8 ng PEG-FGF per gel into the cornea of *Flk1-myr::mCherry* transgenic mice as previously described (5).

Results: The bioactivity of PEG-PDGF was confirmed via increased 10T1/2 proliferation 48 hours after seeding cells on surfaces modified with PEG-RDGS and PEG-PDGF as compared to PEG-RGDS alone (p=0.015). HUVECs seeded on modified surfaces showed significantly more tubule formation on surfaces modified with growth factors as compared to surfaces modified with PEG-RGDS alone (p<0.01; Fig 1). HUVECs encapsulated into degradable hydrogels showed significantly higher migration in gels with covalently immobilized growth factors (p=0.01; Fig 2). In vivo vascular response to hydrogels incorporating both releasable and covalently immobilized PDGF-BB and bFGF (Fig 3 A-B), a previously established synergistic combination (6), showed a more robust vascular response than hydrogels with releasable and covalently immobilized VEGF (C-D). Furthermore, the different growth factors resulted in different vasculature morphologies, where hydrogels with PDGF and FGF induced larger diameter and more organized vessels.

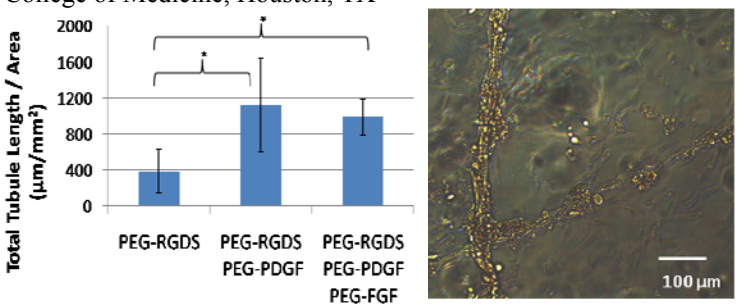

Figure 1: Total Tubule Formation on Modified Surfaces (*p<0.01)

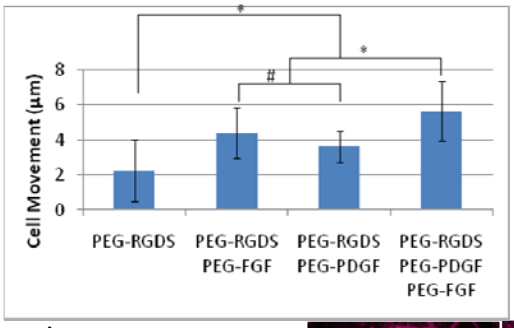

Figure 2: HUVEC migration per hour in 3D degradable hydrogels (*p < 0.01, #p <0.05).

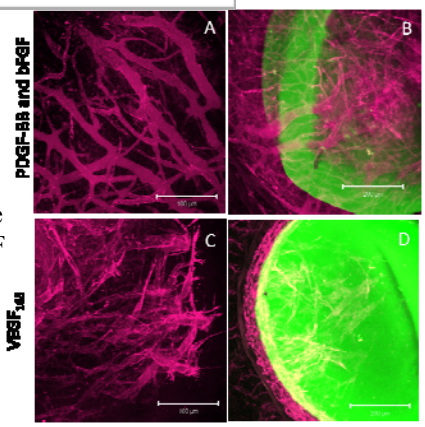

Figure 3: I*n vivo* vascular response to degradable hydrogels with both releasable and covalently immobilized factors.

Conclusions:
Covalently immobilized PDGF promotes angiogenic activity, including endothelial cell migration and tubule formation. The *in vivo* vascular response in transgenic mice induces neovascularization, which can be regulated using rational design of bioactive hydrogels. Based on these results, bioactive hydrogels can be utilized to improve the formation of functional microvasculature for tissue engineering.

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Acknowledgements:
Thank you Daniel Gould and Duncan Shepard for assisting with data analysis and to my funding sources, NSF GRF and NIH P20 EB007076.