Sequential Growth Factor Delivery within Fibrin Loaded Porous Degradable Hydrogels

Bin Jiang, Banu Akar, Thomas M. Waller, Jeffery C. Larson, Alyssa A. Appel, Eric M. Brey
Department of Biomedical Engineering, Illinois Institute of Technology, Chicago, Illinois.

Statement of Purpose: Proper microvascular network formation is essential for engineered tissues. Vascularization occurs via a complex temporal delivery of proteins. Growth factors such as VEGF and FGF are increased at early times to accelerate new blood vessel formation, while maturation factors, such as PDGF-BB and Ang-1, are present at later stages for vessel stabilization.[1] The goal of this study is to develop a biomaterial system that can deliver two growth factors with distinct kinetics, while providing structural and mechanical support for tissue regeneration.

Methods: Poly (ethylene glycol)-co-poly (L-lactic acid) diacrylate (PEG-PLLA-DA) was synthesized as described previously.[2] PLGA microspheres encapsulated with PDGF-BB was prepared with a double emulsion process.[3] A salt-leaching technique was used to synthesize porous PEG-PLLA-DA/PEG-DA hydrogels (300~500μm pore size) with PLGA microspheres and thrombin. Fibrinogen (Fg) solution mixed with FGF-1 and heparin was loaded in pores to where thrombin initiated polymerization of Fg to fibrin.[4] Hydrogel degradation was tested by measuring hydrogel wet weight at 37°C, with varying copolymer and PLGA microspheres concentration. The release kinetics of PDGF-BB and FGF-1 from fibrin in pores were measured with ^{125}I radio-labeled growth factor. A rodent subcutaneous model [4] was used to evaluate hydrogel degradation and tissue response *in vivo*. Hydrogels with different degradation rates (fast, medium and slow based on *in vitro* degradation) and different growth factors (FGF-1 +/-, PDGF-BB +/- and the combination of the two) were implanted against muscle under dorsal skin of rats, and harvested after 1, 2 and 4 weeks. Tissue response was evaluated with histological and immunohistochemical staining.

Results: The PEG-PLLA-DA hydrogel is auto-fluorescent [2] and thus can be visualized using confocal microscopy. (Fig 1.A) PLGA microspheres (mixed with PKH-26) could be identified throughout the hydrogels (Fig 1.B), while fibrin (mixed with Alexa Fluor 647 Fg) was loaded in pores (Fig 1.C) The degradation rate of the hydrogels can be controlled by varying the ratio of PEG-PLLA-DA to PEG-DA, with degradation time ranging from less than 1 week to over 7 weeks. (Fig 2. A) The incorporation of PLGA microspheres accelerated to degradations. (Fig 2.B) The release of PDGF-BB from microspheres showed sustained growth factor delivery, with difference in release kinetics based on hydrogel degradation rates. (Fig 3.A) FGF-1 exhibited rapid release from the fibrin gel within 3 days, with no significant difference found with Fg concentration. (Fig 3.B) Preliminary animal study showed hydrogel degradation rate *in vivo* was similar to *in vitro*. By week 1, fast degradable hydrogels were completely degraded (Fig 4.A), while medium degradable hydrogels were partially degraded (Fig 4.B) and slow degradable hydrogels were mostly intact (Fig 4.C). Further histological and vascular analysis are being performed for evaluating the effect of growth factors delivery to tissue invasion and vascular formation in vivo.

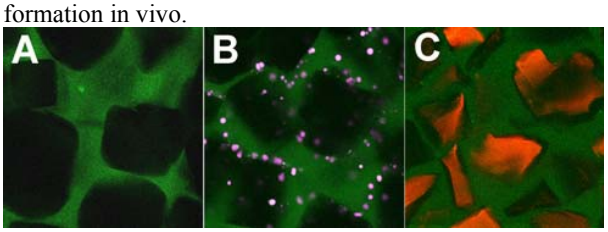

Figure 1. Confocal images of porous hydrogel (A, green) with PLGA microspheres (B, purple) and fibrin (C, red).

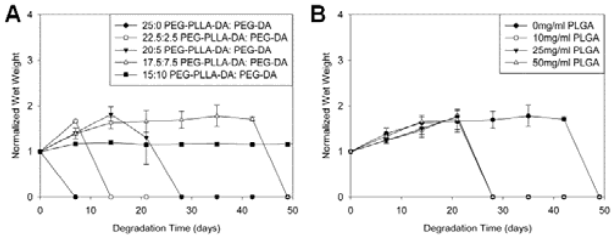

Figure 2. Hydrogel degradation with varying polymer ratio (A) and PLGA microspheres concentration (B).

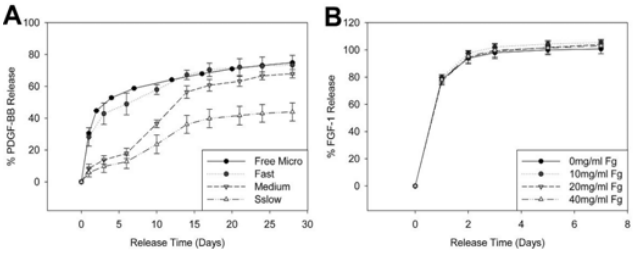

Figure 3. PDGF-BB (A) and FGF-1 (B) release from PLGA microspheres and fibrin incorporated hydrogels.

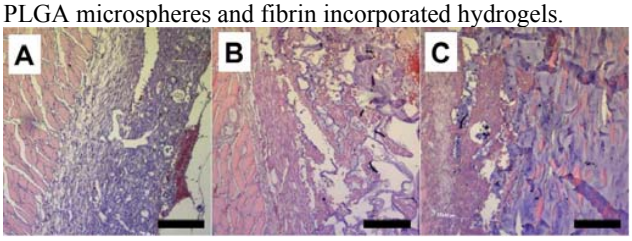

Figure 4. H&E staining of fast (A), medium (B) and slow (C) degradable hydrogels harvested 1 week after implantation. Scale bar=200μm.

Conclusions: We described a biomaterial system with a degradable scaffold for structural support, PLGA microspheres for later stage drug delivery, and fibrin for earlier stage drug delivery, which can be used for vascular tissue engineering applications.

References:

[1] Jiang B and Brey EM, *InTech*. 2011. 477-502.
[2]Chiu YC et al., *J Fluoresc*. 2012;22(3):907-13.
[3] Jiang B et al., *Acta Biomater*. 2012; in press.
[4] Jiang B et al., *Tissue Eng Part A*. 2012 ; in press.