Degradable Hydrogels to Control MSC Localization and Persistence at Decellularized Bone Allografts

Michael D. Hoffman[1,2], Amy H. Van Hove[1], and Danielle S.W. Benoit[1-3]

1-Department of Biomedical Engineering, University of Rochester, 2-Center for Musculoskeletal Research, University of Rochester Medical Center, 3-Department of Chemical Engineering, University of Rochester

Statement of Purpose: The ability to transplant and control cell localization and persistence for regenerative medicine applications faces significant challenges. Direct injection of cells to injury sites is limited by cell migration into surrounding tissue, and cell seeding of preformed materials such as Gelfoam or poly(lactide-co-glycolide) suffers from irreproducible cell attachment, survival, and migration [1]. Here, we investigated hydrogel-mediated transplantation of mesenchymal stem cells (MSCs) surrounding bone allografts to emulate the periosteum, a highly cellularized tissue adjacent to bone that is crucial for healing [2]. Utilizing a segmental femoral defect model, we investigated how the degradation rate of poly(ethylene glycol) (PEG) hydrogels can be altered to control transplanted MSC localization and persistence at allograft surfaces.

Methods: To control MSC localization and persistence at allograft surfaces, hydrolytically-degradable poly(lactide)-b-PEG-b-poly(lactide) dimethacrylate ($PEGPLA_xDM$) tri-block copolymers with complete degradation over ~7 days (x=4) and ~14 days (x=3) were synthesized [2, 3]. Hydrogels formed from these macromers were characterized using compressive modulus and mass loss analysis. Green fluorescent protein expressing mouse MSCs (GFP^+ mMSCs; 500,000 cells/graft to mimic native periosteum cell densities [1]) were encapsulated within hydrogels around allografts using custom molds to ensure uniform annular coatings [2]. Modified allografts were cultured *in vitro* and *in vivo* by implantation into murine femoral segmental defects. GFP^+ mMSC localization and persistence was evaluated in both cases using longitudinal fluorescent imaging. In addition, histology and laser capture microdissection were used to assess transplanted MSC tissue localization and phenotype after complete hydrogel degradation *in vivo*.

Results: $PEGPLA_3DM$ and $PEGPLA_4DM$ hydrogels were synthesized and formed around allografts to encapsulate GFP^+ mMSCs, to emulate the periosteum in its capacity to orchestrate graft healing [1]. Using longitudinal fluorescent imaging, MSC localization to allograft surfaces was analyzed and shown to be in agreement with hydrogel degradation kinetics *in vitro* and *in vivo* [1-2] (Fig. 1A). MSC signal was detectable when transplanted *in vivo* within $PEGPLA_3DM$ and $PEGPLA_4DM$ hydrogels for ~14 and 7 days, a significant increase over MSCs directly seeded onto allograft controls (<1 days, Fig. 1B) [4]. Although hydrogel mesh size (~ 20-30 nm) exceeded MSC size (~ 10 μm) only after complete hydrogel degradation, cell signal at allograft surfaces decayed in close agreement with network degradation kinetics (cell persistence: k' ~ 0.18, ~ 0.29 $days^{-1}$, Fig. 1C; red; hydrogel degradation: k' ~ 0.15, ~ 0.24 $days^{-1}$, Fig. 1C; blue). This may be due to cell apoptosis and/or migration enabled by cell-mediated degradation processes, as previously described for similar hydrogels [5]. Histology and laser capture microdissection performed 7 weeks after complete network degradation demonstrated that MSCs were still in close proximity to defects and exhibited a retained mesenchymal phenotype, indicating that cell migration, rather than apoptosis likely governs reductions in graft-localized MSCs. In fact, quantification of cell number via histological sections showed that ~10% of the transplanted MSCs remained in these areas, an ~ 100-fold increase over direct cell injection methods (~ 0.1%) [6].

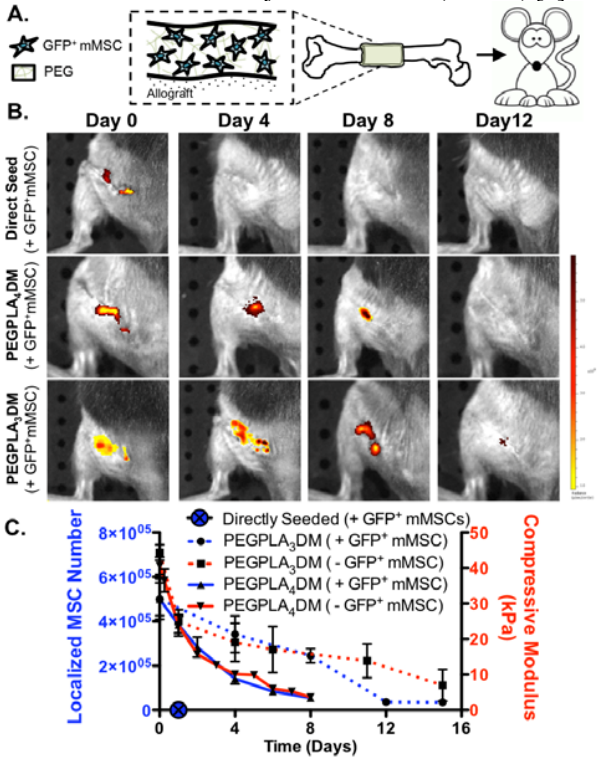

Figure 1. Degradable PEG hydrogels were utilized to transplant MSCs to the surface of bone allografts (A) and *in vivo* cell localization (B) was shown to be in agreement with hydrogel degradation kinetics (C).

Conclusion: Degradable PEG macromers can be modified to alter the temporal localization and persistence of MSCs *in vivo*. These results demonstrate that MSC transplantation via degradable PEG hydrogels provide a reproducible and tunable approach for cell delivery. Current efforts are focused on elucidating the mechanism of cell-dictated degradation and migration from implanted periosteum mimetics. This biomaterials-based methodology can be applied in numerous cell transplantation strategies in regenerative medicine.

References: 1-X. Zhang, *et al*. *JBMR* 2005. 20:2124**,** 2-M.D. Hoffman, *et al*. *Biomaterials*. 2013. 34:8887, 3-A.S. Sawhney, *et al*. *Macromolecules*. 1993. 26:581, 4-A.T. Metters, *et al*. *Polymer*. 2000. 41:3993, 5-C.R. Nuttelman, *et al*. *Adv Exp Med Biol*. 2006. 585:135 , 6-G.E. Zhang, *et al*. *Tissue Eng*. 2008. 14:1025.