MSC Localization via Tissue Engineered Periosteum Mimetics Coordinates Remodeling of Bone Allografts

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Statement of Purpose: The periosteum is critical in autograft healing; its removal, as in the case of allografts, results in impaired graft integration and eventual graft failure (1, 2). Of key importance are periosteal stem cells (PCs) that reside within this thin layer of tissue. PCs have been shown to persist at autografts for a finite period (only ~3 weeks) during the healing process. In an effort to initiate cell-mediated allograft healing and intergation, we developed degradable poly(ethylene glycol) (PEG) hydrogels to use as periosteum mimetics to control cell localization and persistence at allografts. MSCs were used as they are therapeutically superior to PCs and are easy to isolate via bone marrow aspirates.

Methods: To control MSC localization at allograft surfaces hydrolytically degradable poly(lactide)-b-PEG-b-poly(lactide) dimethacrylate (PEG-PLA$_x$-DM) tri-block copolymers with varied degradation kinetics were synthesized by altering the number of lactide repeats per macromer (x = 4, 3, and 1 respectively). Kinetics of degradation of PEG-PLA$_x$-DM hydrogels were verified by compressive modulus and mass loss analysis. Hydrogel precursors were mixed with 500,000 cells/graft of green fluorescent protein (GFP) expressing MSCs to mimic native periosteum cell densities. Hydrogels were photopolymerized *ex vivo* around allografts using custom molds to ensure uniform coating of hydrogel periosteum mimetics. Modified allografts were implanted into mouse femur segmental defects. Transplanted GFP-MSC localization was monitored using live animal imaging. Bone integration/healing was assessed over 9 weeks using microcomputed tomography, torsion testing, and histology. To determine MSC contributions to healing, immunohistochemical labeling was performed to assess MSC localization, proliferation, and apoptosis. Laser capture micro-dissection was also used to analyze potential MSC differentiation.

Results: Our results demonstrate that while PEG-PLA-DM periosteum mimetics can be used to control MSC localization at allografts, sufficient cell delivery, rather than persistence is crucial to coordinate allograft healing and integration. Specifically, quantification of GFP-MSC localization transplanted using hydrogel-based periosteum mimetics resulted in ~3.1-fold greater cell densities immediately after implantation, as compared to allografts directly seeded with GFP-MSCs. Furthermore, GFP-MSC persistence was shown to be governed by hydrogel degradation kinetics. Networks comprised of PLA$_4$, PLA$_3$, and PLA$_1$ functionalized macromers resulted in ~7, 12, and 21 day (k' = 0.55, 0.27, and 0.18 hr^{-1}) GFP-MSC persistence times, respectively, as predicted by previously established models (3), and statistically different than direct seed controls (~3 days). Despite demonstrating significant changes in degradation kinetics and subsequent MSC persistence between hydrogel compositions, changes in healing effects were not observed at 6 or 9 weeks regardless of overall MSC persistence times. Periosteum mimetics comprised of PLA$_3$ and PLA$_4$ functionalities exhibited statistically similar ~1.9-fold and ~2.0-fold respective increase in bone callus volume as compared to allograft only and directly seeded controls at 9 weeks. Furthermore, both networks results in ~3.5-fold increased vascular infiltration compared to allograft only and directly seeded controls. These results suggest that enhanced healing, mediated by hydrogel localization is likely a result of MSC paracrine factor release initiating host cell recruitment and remodeling rather than direct participation of the transplanted MSC population.

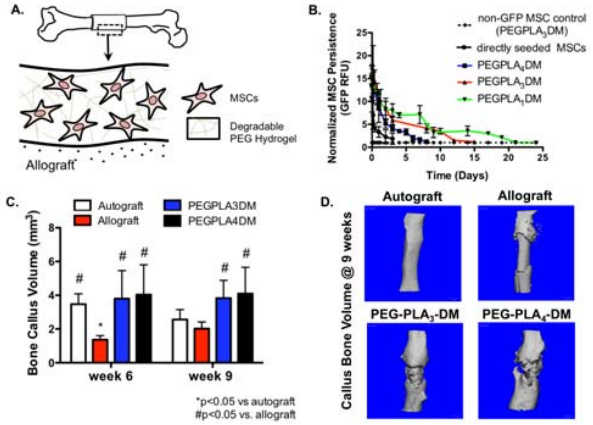

Figure 1. PEG hydrogel based periosteum mimetics (A) control GFP-MSCs persistence at the allograft surface (B) and coordinate increased remodeling and bone callus formation (C & D) compared to allograft only controls.

Conclusions and Future Directions: Herein we demonstrate that degradable hydrogels can be used to control MSC localization to allograft surfaces in vivo. Furthermore, we show that delivery of MSCs via degradable hydrogels enhances healing and integration, independent of MSC persistence, as compared to both allograft only controls as well as directly seeded MSCs (no hydrogel carrier). It is hypothesized that no relationship between MSC persistence and healing is observed because substantial paracrine factor release immediately following implantation results in robust host cell recruitment and subsequent initiation of remodeling. We believe that host-cell effects, rather than persistence MSC localization is the key factor driving enhanced periosteum mimetic coated allograft healing and integration. To examine this hypothesis we are currently investigating the role MSC released vascular endothelial growth factor (VEGF) plays in host-mediated vascularization and presumed osteoclast activity.

References: 1-Zhang, X., et al. *J Bone Miner Res*. 2005, 20:2124**,** 2-Xie, C., et al. *Tissue Eng*. 2007, 13:435**,** 3-Metters, A., et al. *Biomed Sci Instrum*. 1999, 33:53