Integrin α2β1-Specific Hydrogels Enhance Human Mesenchymal Stem Cell Survival and Bone Repair in Vivo

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Statement of Purpose: Cell-based strategies have emerged as promising therapies for the treatment of musculoskeletal defects. Adult human mesenchymal stem cells (hMSC) have shown promising results in clinical trials, but inadequate control of cell survival, engraftment and fate limits the success of this cell-based therapy. Integrin-mediated cell adhesion plays a central role in tissue formation, maintenance, and repair by providing anchorage forces and triggering signals that regulate cell function. We hypothesize that biomaterials presenting integrin-specific adhesive motifs will direct hMSC signaling and specification. The objective of this project is to engineer bioartificial hydrogels presenting integrin-specific ligands to create biomimetic niches for hMSC differentiation as well as cell delivery vehicles for enhanced in vivo engraftment and function. This research is innovative because it focuses on engineering specificity to integrin receptors to promote stem cell differentiation and survival without the use of exogenous growth factors, integrates novel in vivo imaging, and utilizes novel hydrogel chemistry.

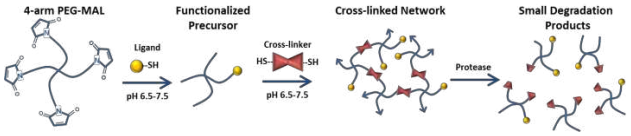

Fig. 1 PEG-maleimide hydrogel system and reaction

Methods: Hydrogel-encapsulated hMSCs were delivered into a non-healing radial segmental defect in mice to evaluate engraftment and differentiation. PEG-maleimide (PEG-MAL) 4-arm macromers were functionalized with one of three ligands: collagen-mimetic GFOGER triple helical peptide, RGD peptide, or scrambled, non-adhesive rDg peptide and cross-linked by addition of cysteine-flanked MMP-degradable peptide sequences [1] (Fig.1). hMSCs were transduced to constitutively express red firefly luciferase (RFLuc) for longitudinal tracking of cell number with high efficiency (>80%). 2.5 mm defects were created in the radii of 8-9 week old male NOD scid gamma mice. Treatment groups included hydrogels functionalized with GFOGER, RGD, or rDg containing 15k hMSCs. Bioluminescence of the transplanted cells was monitored using an In Vivo Imaging System (IVIS, PerkinElmer). New bone formation was evaluated using an in vivo microcomputer tomography scanner (Viva CT40, Scanco Medical).

Results: PEG-mal reaction chemistry allows for rapid gelation with high cytocompatibility while still allowing "plug-and-play" design variation [2]. Bioluminescence of transplanted RFLuc-hMSCs was monitored after transplantation (Figure 2A). Figure 2B shows that GFOGER-functionalized hydrogels support the proliferation of the transplanted cells in vivo whereas the bioluminescent signals for hMSCs delivered in a RGD- or rDg-functionalized hydrogel remained low.

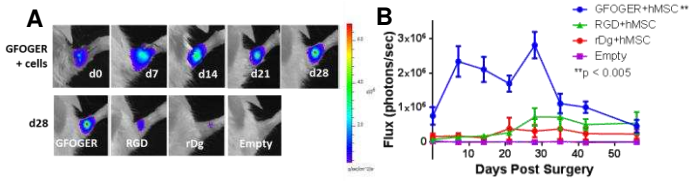

Fig. 2: α2β1 integrin–specific GFOGER enhances hMSC survival in vivo compared to RGD. Bioluminescence was monitored using IVIS at various time points throughout the 8 week study. A) IVIS images. B) Luminescent signal quantified and reported as photon flux. ** $p < 0.005$ by two-way ANOVA.

Unmodified hMSCs were transplanted into the segmental defect and bone volume was monitored by microCT (Figure 3). $\alpha_2\beta_1$ integrin–specific GFOGER enhances bone repair in vivo compared to RGD and rDg with no statistical difference between groups receiving genetically modified hMSCs expressing RFLuc and unmodified hMSCs (Figures 3A, B).

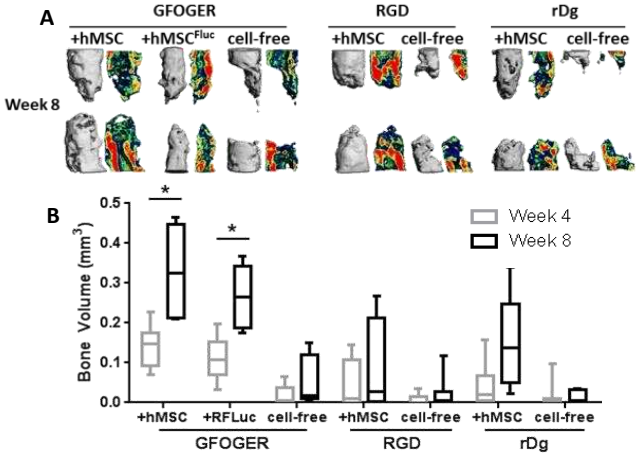

Fig. 3: α2β1 integrin–specific GFOGER enhances bone repair and hMSC viability in vivo. A) MicroCT reconstructions with sagittal mineral density maps at 8 weeks. B) Bone volume at weeks 4 and 8. * $p < 0.05$ by two-way ANOVA and Tukey's multiple comparisons test, whiskers represent min and max values.

Conclusions: We have demonstrated that this system allows for the longitudinal tracking of cell number and bone formation for the delivery of hMSC in a novel biomaterial and supports bone formation in a non-healing defect. GFOGER-functionalized PEG hydrogels support bone formation and hMSC viability in vivo compared to RGD and rDg. These studies are ongoing for immunohistochemical analyses. This work highlights the importance of integrin-specificity in cell delivery for engraftment and tissue repair.

References:

1. Phelps EA, Adv Mat 2012, 24(1):64-70
2. Phelps EA, Biomaterials 2013, 34(19):4602-11

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