Integrin-Specific Hydrogels for the Delivery of Human Mesenchymal Stem Cells in Bone Repair

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Statement of Purpose: Cell-based strategies have emerged as promising therapies for the treatment of diseased organs. Adult human mesenchymal stem cells (hMSC) constitute a critical component of the hematopoietic stem cell niche in the bone marrow, and although hMSCs have shown promising results in clinical trials, inadequate control of cell fate and cell engraftment in host tissues 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. The following 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.

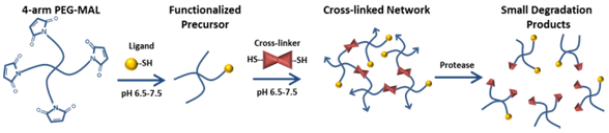

Fig. 1 PEG-maleimide hydrogel system and reaction

Methods: Hydrogel-encapsulated hMSCs were delivered into a radial segmental defect of mice to evaluate engraftment and differentiation. PEG-maleimide (PEG-MAL) 4-arm macromers were functionalized with one of three ligands: collagen-mimetic GFOGER, RGD peptide adhesion 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 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 (Jax) mice. A perforated polyimide tube was fit over the ends of the bone in the defect. Treatment groups included hydrogels functionalized with GFOGER, RGD, or rDg cell-free ("GFOGER", "RGD", "rDg") or containing 30k hMSCs ("GFOGER + cells", "RGD + cells", "rDg + cells"). Bioluminescence of the transplanted cells was monitored using an In Vivo Imaging System (IVIS, PerkinElmer) at days 0, 3, 7, 14, 21, and 28. New bone formation was evaluated using an *in vivo* microcomputer tomography scanner (Viva CT40, Scanco Medical) at week 4. This study is ongoing.

Results: The engineered bio-functionalized PEG matrix with maleimide cross-linking reaction chemistry gels rapidly with high cytocompatibility while still allowing "plug-and-play" design variation [2]. Figure 2 shows the results through week 4 of the ongoing 8 week study. Figure 2A shows that GFOGER 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. Bone volume measured by microCT is shown in Figure 2C. GFOGER + cells and GFOGER hydrogels resulted in significantly higher bone volumes compared to RGD or rDg hydrogels with or without cells. Representative IVIS and microCT images with sagittal mineral density maps are shown in figures 2C and 2D, respectively.

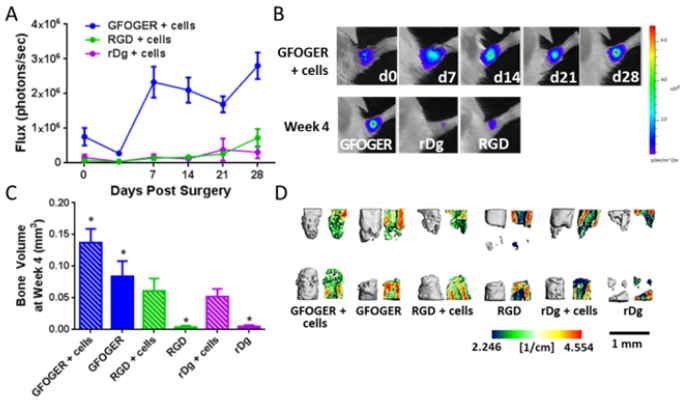

Fig. 2 GFOGER-hydrogel supports transplanted hMSC proliferation *in vivo* and bone repair. A) Bioluminescence as measured by IVIS at days 0, 3, 7, 14, 21, and 28. $p < 0.0001$ by two-way ANOVA. B) Volume of newly formed bone measured by micro CT at week 4. $p < 0.0001$ by one-way ANOVA. C) Representative IVIS images at week 4. D) Representative microCT images and sagittal density maps at week 4.

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. This study is ongoing for immunohistochemical analysis. 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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