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 for bone repair, 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 hypothesized that biomaterials presenting integrin-specific adhesive motifs will direct hMSC signaling and specification. Our objective was 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, integrates novel in vivo imaging, and utilizes novel hydrogel chemistry.

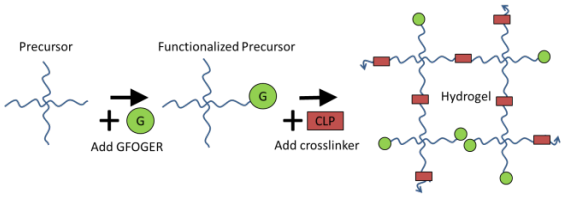

Fig. 1 PEG-maleimide hydrogel system and reaction

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 collagen-mimetic GFOGER adhesion peptide and cross-linked into a hydrogel by addition of cysteine-flanked MMP-degradable peptide sequences (Fig.1). hMSCs were transduced to constitutively express red firefly luciferase for longitudinal tracking of cell number. 2.5 mm defects were created in the radii of male NOD.SCID mice. A perforated polyimide tube was fit over the ends of the bone in the defect. Treatment groups included an empty tube ("empty"), cell-free GFOGER hydrogel ("cell-free"), 2.5 x 10^4 hMSCs in GFOGER hydrogel ("low"), or 5 x 10^4 hMSCs in GFOGER hydrogel ("high"). Bioluminescence of the transplanted cells was monitored using an In Vivo Imaging System (IVIS, PerkinElmer) at days 0, 4, 7, 14, 21, and 28. Bone formation was evaluated using an in vivo microcomputer tomography scanner (Viva CT40, Scanco Medical) at weeks 4 and 8.

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 [1]. Fig. 2 shows the results of the 8-week pilot study. Bioluminescence was detectable up to 3 weeks for the high cell dose group, but the signal for the low-dose group was too low to detect (Fig. 2A). As expected, the cell-free and empty tube groups showed no luminescence. The low signal from the low cell-dose group may be due to the transduction efficiency as only 50% of the transplanted cells expressed luciferase. Fig. 2B shows the bone formation for each animal at week 8 as measured by micro CT. The empty tube showed little to no bone formation, while the low cell dose, high cell dose and cell-free dose supported bone formation. No significant difference is noted in the bone formation of newly formed bone. Representative IVIS and microCT images are shown in figures 2C and 2D, respectively.

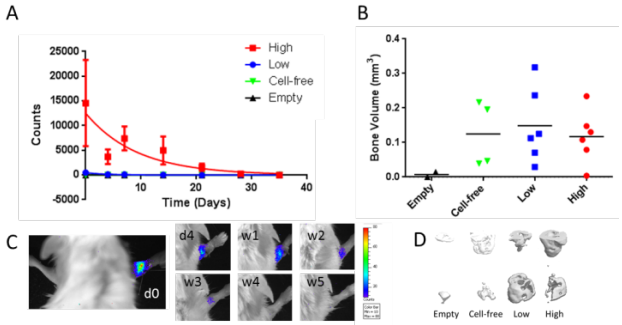

Fig. 2 Bone formation and bioluminescence. A) Bioluminescence as measured by IVIS at days 0, 4, 7, 14, 21, and 28. Error bars represent standard error of the mean. B) Volume of newly formed bone measured by micro CT at week 8. C) Representative IVIS images of a bioluminescence in a medium cell dose at various time points. D) Representative micro CT images of the middle 2 mm of defect at week 8.

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. The GFOGER-functionalized PEG hydrogel supports bone formation and hMSC viability *in vivo*. Bioluminescent signals were detectable for up to 3 weeks at 50% transduction efficiency. This study is ongoing for immunohistochemical analysis. Future studies are aimed at increasing luciferase expression by sorting before transplantation and increasing sample size.

References:

1. Phelps EA, *Advanced Materials.* 2011, 24:64-70

This work was funded by the NIH (R01 AR062368). AC was supported by the NIH Cell and Tissue Engineering Training Grant (T32 GM008433) and the NSF IGERT on Stem Cell Manufacturing (DGE 0965945).