Osteogenic differentiation of hMSC in PEG diacrylate/hyaluronic acid semi-IPNs

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Statement of Purpose: Hybrid hydrogels are designed to integrate the precise control of composition/properties provided by synthetic networks with the bioactivity of naturally-derived materials. PEG derivatives crosslinked with MMP-sensitive peptides are a prominent example and have been widely used for *in vitro* culture models and *in vivo* tissue engineering. Alternatively, the use of intact, naturally-derived macromolecules offers several benefits including substantially lower cost and preservation of native structure supporting higher rates of enzymatic degradation. Previously, we have shown that incorporation of native hyaluronic acid (HA) into photocrosslinked networks of hydrolytically degradable PEG diacrylates (PEGdA) creates semi-interpenetrating networks (semi-IPNs) that support increased cell spreading and proliferation relative to fully synthetic networks that is dependent on cellular hyaluronidase activity.[1,2] In recent work, we have optimized network composition (HA concentration, blending PEGdAs with various hydrolytic degradation rates) to obtain networks that support prolonged cellular remodeling. In addition, these networks possess elastic moduli values (~10 kPa) previously reported as optimal for osteogenic differentiation in three-dimensional (3D) culture.[3] The objective of this study was to investigate the ability of these semi-IPNs to serve as matrices for 3D hMSC culture and osteogenic differentiation.

Methods: Three PEGdAs with varying susceptibility to hydrolytic degradation were synthesized by a two-step process. First, PEG (Fluka, MW 4000) was reacted with chloroacetyl chloride, 2-chloropropionyl chloride, or 4-chlorobutyryl chloride and then the resulting intermediate products were reacted with sodium acrylate to obtain PEG-bis-AA, PEG-bis-AP, or PEG-bis-AB.[2] Human mesenchymal stem cells (hMSC, Lonza) were encapsulated (12.5 million cells/mL) in photo-crosslinked PEG-dA/HA semi-IPNs [6% w/v PEGdA blend (12.5% PEG-bis-AA, 37.5% PEG-bis-AP, and 50.0% PEG-bis-AB)] with 0.36% w/v HA (1500 kDa) and 1μmol/mL of acrylate-PEG-GRGDS peptide and cultured in the presence of osteogenic supplements (50 μM ascorbic acid, 10 mM β-glycerophosphate, and 100 nM dexamethasone). LIVE/DEAD (Invitrogen) assay was used to check initial cell survival ratio (at 24 hour) for different polymerization conditions (time and photoinitiator concentration) along with crosslinking properties obtained from mass swelling ratio. After 7, 14, 21, 28 and 35 days in culture, hydrogel samples were fixed and stained with Alexa Fluor 647 phalloidin (Invitrogen) for confocal microscopy (Nikon Ti Eclipse). Cell morphology at 200 μm inside the gel was compared. Other fixed samples were cryosectioned for von Kossa and Alizarin red S stain to show calcium deposition. Quantitative calcium assay was also performed using atomic absorption spectrometry (Perkin Elmer) with two negative control groups (blank hydrogel without cells and cell encapsulated hydrogel in osteogenic media without dexamethasone).

Results: At 24 hours post-encapsulation, more than 75% of hMSCs remained viable. Cell spreading was supported in selected semi-IPNs and could be observed till day 28. At later stage of culture (day 35), confocal scanning was hindered by deposited calcium. Both von Kossa and Alizarin red S staining showed a gradual increase in calcium deposition till day 21 and substantial increase after 21 days of culture. This trend (increase in calcium deposition at later stage) was also confirmed by quantitative data from AA spectrophotometry. As shown in Figure 1, calcium deposition was observed throughout the entire volume of hydrogel by day 35. Acellular control hydrogels showed little, but obvious calcium deposition, suggesting that non-physiological calcium deposition was still possible with full osteogenic media. Cell encapsulated hydrogels in osteogenic media without dexamethasone showed higher calcium deposition than acellular hydrogels, but significantly less calcium contents compared to hydrogels in full osteogenic media, indicating that semi-IPN mineralization was primarily attributable to dexamethasone-induced hMSC differentiation.

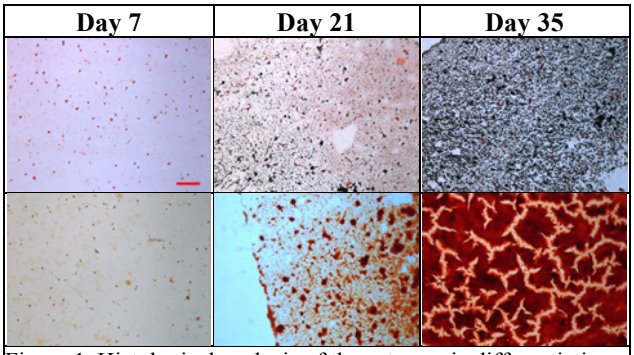

Figure 1. Histological analysis of the osteogenic differentiation of hMSC encapsulated semi-IPNs at various time points. Top panel: von Kossa staining with Safranin O counter stain, Bottom: Alizarin red S staining, bar = 100 μm.

Conclusions: PEGdA blend/HA semi-IPNs supported initial hMSC survival, spreading, and long term osteogenic differentiation. HA enriched zone from phase separation between the two components occurring during photocrosslinking provided the space for initial cell spreading via enzymatic degradation. The orchestrated prolonged degradation resulted in physiological calcium deposition during hMSC differentiation and network remodeling over 35 days in culture.

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References: [1]Kutty JK. Biomaterials 2007;28:4928-38. [2]Cho EH. J Biomed Mater Res. 2009;90A:1073-82. [3]Huebsch N. Nat Mater. 2010;9;518-26.