Development and Characterization of Composite PEG Hydrogels for Osteochondral Tissue Engineering

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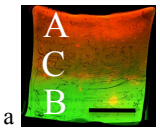
Statement of Purpose: Photopolymerized poly(ethylene glycol) (PEG) based hydrogels are promising scaffolds for osteochondral tissue regeneration due to the ease with which the scaffold properties can be tuned[1]. Our research focuses on designing a 3D composite scaffold with a characterized gradient chemistry to direct both osteogenic and chondrogenic differentiation of encapsulated human mesenchymal stromal cells (hMSCs). In this study, we explore two key features in designing spatial property gradients in a composite scaffold that will support both osteogenic and chondrogenic MSC differentiation: i) varying the mechanical properties within the scaffold and ii) covalent incorporation of key biochemical moieties (chondroitin sulfate (CS)[2] and P-15 peptide[3]).

Materials and Methods: hMSCs were isolated from the marrow of a single male donor via hMSC selective attachment to tissue culture treated polystyrene. Poly(ethylene glycol) dimethacrylate (PEGDM) macromer was dissolved in basal media with 0.05% (w/w) photoinitiator (I2959, Ciba Specialty Chemical). For chondrogenic regions, 10% (w/w) PEGDM and methacrylated chondroitin sulfate were combined at 80% PEGDM and 20% (w/w) CS (80/20 PEGDM-CS) using a cell concentration of $5x10^6$ cells.mL^{-1}. For osteogenic regions, 30% (w/w) PEGDM and 2.8mM monoacrylated P-15 peptide were combined using a cell concentration of $25x10^6$ cell.ml^{-1}. Passage 4 hMSCs were trypsinized, pelleted, and then resuspended in one of the various macromer solutions described and polymerized under 365 nm light at ~2 mW.cm^{-2}. Osteogenic regions were polymerized for 1 minute and then the chondrogenic region was added and the composite was polymerized for 10 additional minutes.

Histological analysis was conducted. 10μm slices of samples were stained by von Kossa for mineralization and counter stained with H&E. Total calcium assay (Stanbio) was used. Data is presented as mean ± standard deviation (n=3) and a confidence level of 0.95 was considered significant.

Results: Challenges exist in engineering osteochondral scaffolds that can support the concomitant differentiation of MSCs down both osteogenic and chondrogenic differentiation pathways toward the goal of developing osteochondral tissue. We explored the possibility of engineering a gradient scaffold that had tunable mechanical properties as well as variable biochemical differentiation moieties (CS and P-15) within a single scaffold. Chondroitin sulfate (CS) is a glycosaminoglycan (GAG) molecule found in the extraceullular matrix (ECM) of cartilage, and P-15 mimics the cell-binding domain of collagen I, the primary collagen in bone ECM.

Figure 1a illustrates the construction of an acellular scaffold with a spatial mechanical property gradient. It is apparent from the mixing of the covalently attached red and green flurophores that the interfacial region (C)



a b

	Compressive Modulus (kPa)
10% PEGDM	33±0.2
30% PEGDM	477±58
10%/30% PEGDM	96±16

Fig. 1 (a) Composite scaffold with a spatial mechanical property gradient. A: 10% (w/w) PEGDM & B: 30% (w/w) PEGDM, and (b) Compressive modulus data for uniform and composite PEGDM scaffolds. Scale bar = 2 mm.

comprises a mixture of properties from regions A and B, as evidenced by the compressive modulus data in Fig 1b.

Cell-laden composite scaffolds incorporating both gradient mechanical properties and biochemical moieties were cultured in basal media. Fig 2a shows mineralization within the osteogenic region (E) of the scaffold on a gross

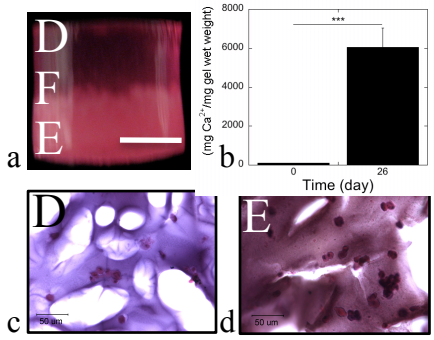


Fig. 2 (a) Cell-laden composite after 21 days of culture; region D: 10% (w/w) 80/20 PEGDM-CS and region E: 30% (w/w) PEGDM/2.8 mM P-15. (b) Total calcium production at day 26. (c&d) von Kossa/ H&E staining at day 21. Darker mineralization staining is present in the osteogenic region (E) (d) as compared to the chondrogenic region (D) (c). Scale bar = 2mm (a), 50 μm (c&d).

scale, in the absence of endogenous differentiation cues, after 28 days. Cells produced a significant amount of total calcium (Fig 2b) after 26 days of culture. Figs 2c and d illustrate that mineralization is observed in the osteogenic region (E) but is absent in the chondrogenic region (D) of the same composite scaffold after 21 days.

Conclusions: We demonstrate the successful construction of composite PEG scaffolds to direct both osteogenic and chondrogenic differentiation of hMSCs by spatially controlling both mechanical properties and biochemical moieties within the composite scaffold. We are currently characterizing the chondrogenic differentiation and the physical/mechanical properties of the scaffolds.

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