Patterned hMSC Differentiation in 3D Hydrogels Based on Network Structures

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Statement of Purpose: Recent efforts in biomaterials research have increasingly focused on the 3-dimensional (3D) interactions of cells and synthetic hydrogels.[1] While much progress has been made in promoting cell/scaffold communication (e.g., the tethering of cells to materials via ligands) through incorporation of physical or biochemical cues, spatially controlled stem cell differentiation has not yet been a major focus. In this work, we demonstrate directed differentiation of human mesenchymal stem cells (hMSCs) based on spreading cues (via controlled network structures and degradability) within 3D hyaluronic acid (HA) hydrogels, including with spatial control. HA was chosen due to its biocompatibility[2], versatility in crosslinking, and common use as a biomaterial.[3,4]

Methods: Acrylated HA (AHA, ~62% acrylation from ^{1}H NMR) was prepared via the coupling of acrylic acid (AA) and the tetrabutylammonium salt of HA (HA-TBA), purification by dialysis, and lyophilization. A uniform hydrogel expected to be permissive to cell spreading (-UV) was first formed using monofunctional, pendant cell adhesive peptides (GCGYG**RGDS**PG) and bifunctional, crosslinking proteolytically degradable peptides (GCRDGPQGIWGQDRCG) to consume 50% of total acrylates. The gelation was performed in the presence of 0.05 wt% I2959 photoinitiator (at this point, non-reactive). The gel was then exposed to 10 mW/cm^2 UV light through a photomask applied directly to the surface, resulting in secondary free radical crosslinking and +UV gel structure only in unmasked regions (Figure 1A). Methacrylated rhodamine (MeRho, 50 µM) was mixed into the prepolymer solution to label +UV regions. For differentiation studies, hMSCs (Lonza, 5 x 10^6 cells/mL) were encapsulated in either uniform or 250 µm stripe patterned 3 wt% AHA hydrogels (cells were re-suspended in pre-polymer solution prior to crosslinking). After two weeks of culture in a 1:1 mixed adipogenic/osteogenic inductive medium, the gels were stained with antibodies for osteocalcin (OC, osteogenic differentiation) and fatty acid binding protein (FABP, adipogenic differentiation) using standard immunostaining protocols.

Results: Laser scanning confocal microscopy (LSCM) images of the top and bottom surfaces of patterned hydrogels (Figure 1A) demonstrates that patterning fidelity, visualized by MeRho incorporation into +UV regions, was retained through the sample depth. As expected, the increased crosslink density in +UV environments was associated with higher mechanical properties (elastic modulus) relative to –UV environments, as measured by atomic force microscopy (AFM) of both uniform and patterned hydrogels (Figure 1B). The degradability of –UV versus +UV environments led to differences in encapsulated cell spreading, based on the ability of a cell to degrade crosslinks, including with spatial control (Figure 1C). These differences were confirmed further by SEM visualization of acellular gels treated with exogenous MMP-2 (data not shown).

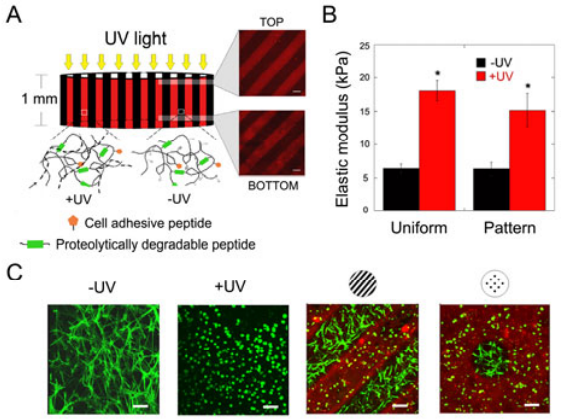

Figure 1. Photopatterning of AHA hydrogels. (A) Schematic of photopatterning of hydrogels; confocal images are the top and bottom surfaces of a gel patterned with 250 µm stripes. Scale bars = 200 µm. (B) Elastic modulus of uniform –UV and +UV hydrogels, and –UV and +UV regions of patterned hydrogels, measured by AFM. * denotes statistically significant difference ($p<0.05$) between conditions for uniform and patterned gels. (C) Calcein (live) staining of encapsulated hMSCs in uniform and patterned gels at 14 d. Scale bars = 100 µm.

Figure 2 illustrates a clear dependence of hMSC differentiation on cell morphology within 3D hydrogels. Biochemical assays indicate primarily osteogenic differentiation when cells spread (-UV) and adipogenic differentiation when cells are round (+UV) (Figure 2A). Representative x-y projections from the interior of either uniform (Figure 2B) or patterned (Figure 2C) hydrogels illustrate that cells in –UV environments stain for OC (osteogenic marker, green), whereas rounded cells stain for FABP (adipogenic marker, blue). Lineage fate within uniform and patterned gels was further confirmed by real-time PCR and histological staining for markers of adipogenesis and osteogenesis (data not shown).

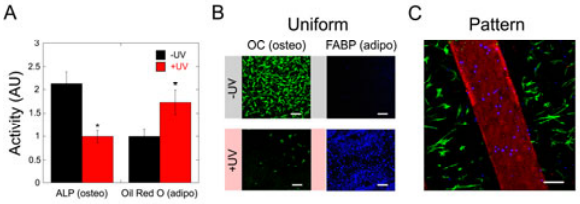

Figure 2. Spatially patterned hMSC differentiation. (A) Quantitative assays for markers of osteogenic (alkaline phosphatase) and adipogenic (oil red O) hMSC differentiation. (B) Representative immunostaining of FABP (blue; adipogenesis) and OC (green; osteogenesis) in uniform –UV and +UV hydrogels; channels shown separately for ease of viewing. Scale bars = 100 µm. (C) Representative immunostaining of the same targets in a hydrogel patterned with 250 µm stripes. Scale bar = 100 µm.

Conclusions: This work demonstrates good spatial control of hMSC differentiation within 3D hydrogels. Gel properties (e.g., adhesive ligand density and elasticity) within –UV and +UV regions may potentially be tuned for different lineages, indicating the potential of this paradigm for tissue engineering applications.

References:
[1] Tibbitt MW. Biotechnol Bioeng. 2009:103:655-83. [2] Prestwich G. Biomaterials. 2005:23:4737-46, [3] Chung C. Tissue Eng. 2006:12:2665-73, [4] Sakai Y. Biomed Mater Eng. 2007:17:191-197.