Patterning 3D Hydrogel Remodeling through Controlled Presentation of Biomimetic Signals

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Statement of Purpose: Recent efforts in biomaterials research have increasingly focused on the 3-dimensional interaction of cells and synthetic hydrogels[1]. While much progress has been made in promoting scaffold interactions (e.g., the tethering of cells to materials via ligands) through incorporation of physical or biochemical cues, spatially controlled matrix remodeling by external or encapsulated cells has not yet been a focus. In this work, we demonstrate common responses of cells in suspension (mesenchymal stem cells), tissue explants (chick aortic arches) and tissue *in vivo* (rat subcutaneous implantation) to synthetic hydrogels based upon the gel structure. Hyaluronic acid (HA) was chosen as the core polymer due to its biocompatibility[2] 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. The (A) structure of AHA and schemes for sequential crosslinking and (B) photopatterning are shown in Figure 1. In the primary step, AHA macromers (first reacted with a biomimetic cell adhesive peptide GCGYG**RGDS**PG, 1mM) underwent an addition reaction with bifunctional, matrix metalloprotease (MMP)-degradable oligopeptides (GCRDGPQGIWGQDRCG) to consume 50% of total acrylates (-UV). Selected gels then underwent a secondary photoinitiated radical polymerization of remaining acrylates with 10 mW/cm^2 UV light and I2959 photoinitiator (+UV). A third group of gels was photopatterned with light exposure through a high resolution mask during the secondary crosslinking step. For encapsulation studies, hMSCs (Lonza, 5 x 10^6 cells/mL) and 1 mm^3 chick aortic arch explants (Charles River Labs, day 12 after fertilization) were encapsulated in 3 wt% AHA hydrogels of compositions described above. After two weeks of culture in growth media, live cells were visualized with calcein. For *in vivo* studies, acellular hydrogels were implanted subcutaneously in rats. At three weeks post-implant, the explanted constructs were fixed, embedded in paraffin and sectioned using standard protocols, and stained for hematoxylin and eosin (H&E).

Results: Hydrogels were synthesized with controlled biomimetic cues through spatially uniform crosslinking to direct the gel structure dependent response of a range of cell types, both *in vitro* and *in vivo* (Figure 2). The (i) "permissive" –UV hydrogels, distinguished by cell adhesivity and proteolytic degradability, were remodeled by the cells or tissue, resulting in robust *in vitro* cellular outgrowth (Figures 2A and 2B) or *in vitro* cell infiltration into the scaffold (Figure 2C), respectively. In contrast, the introduction of covalent, non-degradable crosslinks and reduced mesh sizes in the (ii) "inhibitory" +UV environments largely restricted encapsulated cell spreading and the remodeling response.

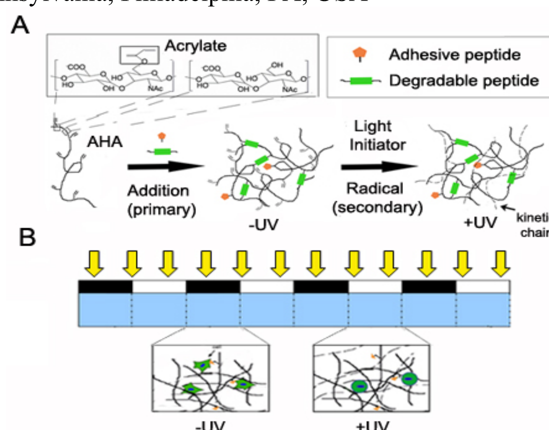

Figure 1. Schematic of (A) sequential and (B) photopatterned crosslinking of AHA hydrogels.

When a mask (400 µm black dots (A) and black semicircle (B, C), respectively) was used to block light over a portion of the gels in the secondary step, the permissive and inhibitory responses were recapitulated in the corresponding –UV and +UV regions of the (iii) photopatterned gels. Though only simple masks were used for the current work, more complex masks or lasers could be used to obtain high resolution features.

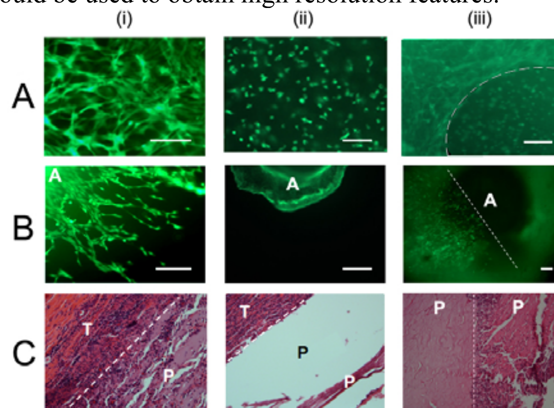

Figure 2. Response of (A) cells in suspension, (B) tissue explants, and (C) tissue *in vivo* to (i) –UV, (ii) +UV, and (iii) photopatterned (pattern outline shown with dotted white line) hydrogel microenvironments. A = arch, T = tissue, P = polymer. Scale Bars = 50 µm.

Conclusions: This work demonstrates good spatial control of 3D hydrogel remodeling based on gel structure and indicates potential value for tissue engineering applications. Because hydrogel properties (e.g., elasticity) that dictate cellular phenotype (e.g., cell spreading, stem cell fate) differ in photopatterned regions of gels, this approach may be useful in the construction of scaffolds capable of supporting multiscale tissue formation. Such techniques could also be used to temporally control matrix remodeling and molecule delivery, by varying the ratio between addition and radical crosslinks.

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.