Fabrication of Advanced Poly(ethylene glycol) Diacrylate Hydrogels for Heart Valve Tissue Engineering

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Statement of Purpose: Heart valve disease is prevalent worldwide, with the number of patients requiring heart valve replacement estimated at over 850,000 in 2050 [1]. Tissue engineered aortic valve has the potential to provide advanced solutions to the limitations of current treatments [2, 3]. In this study, advanced poly(ethylene glycol) diacrylate (PEGDA) hydrogels incorporated with bioactive peptides were patterned to mimic anisotropic mechanical behavior of aortic valve leaflets and their biological functions. Moreover, micro-patterns of RGDS were used to modulate single cell size and morphology, which influenced activation of valvular interstitial cells (VICs) in vitro.

Methods: The acrylation degree of PEGDA was characterized using ^{1}H NMR. Stripe-pattern (thickness ~20, 50 and 100 μm) hydrogels and arrays of RGDS micro-patterns (i.e. rectangles and ovals at different sizes and aspect ratios) were prepared by crosslinking 3.4 kDa PEGDA and 3.8 kDa PEG-RGDS, respectively, to a 20 kDa PEGDA hydrogel slab through photolithographic patterning with pre-designed photomasks. Mechanical properties of these hydrogels were measured by uniaxial compression and tension tests, as well as three-point bending. Bioactive peptides (cell-adhesion peptide RGDS and matrix metalloproteinase (MMP)-sensitive peptide "PQ") were incorporated into the PEGDA hydrogel network. Interaction of primary VICs with these biomimetic PEG-peptide hydrogels was investigated in both two-dimensional (2D) and three-dimensional (3D) cultures. Effects of the amount of RGDS (0.3-5 mM) and PQ (3%, 5% and 10%) on VIC spreading and production of new extracellular matrix proteins as well as hydrogel degradation were investigated. Secretion of MMPs for hydrogel degradation was examined by the zymography test, while new extracellular matrix proteins were stained by immunofluorescence staining.

Results: The physical and mehcanical properties of PEGDA hydrogels were dependent on the molecular weight and concentration of pre-polymer solution as well as crosslinking time. For example, 10% (w/v) 20 kDa PEGDA hydrogels swelled up to 3 times of their original weight and had an average tensile modulus ~6 kPa, while 10% (w/v) 3.4 kDa PEGDA hydrogels rarely swelled and had an average tensile modulus of 52 kPa. Several stripe-patterned hydrogels showed significantly different modulus when tested parallel and perpendicular to the stripe pattern under tension (e.g. 10%, 15% and 20% samples in Fig. 1) and bending condition (anisotropic behavior). Biomimetic PEG-peptide hydrogel with 5 mM RGDS and 5% (or 10%) PQ was found to support cell adhesion and spreading, but not PEGDA hydrogel alone. New extracellular matrix proteins, such as type I collagen and fibronectin, were found to be secreted by cells cultured in PEG-peptide hydrogels (Fig. 2). Additionally, secretion of MMP2 was confirmed in cell culture on PEG-peptide hydrogels by the zymography test (data not shown), suggesting potential degradation of these hydrogels by VICs.

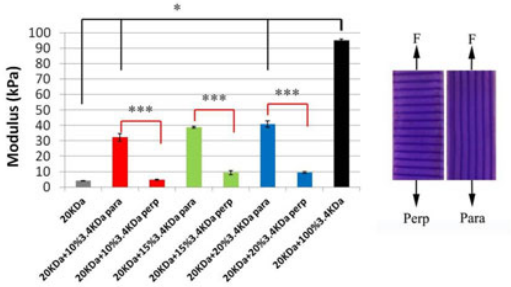

Fig. 1. Tensile stiffness from stripe-patterned hydrogels, stained with cresyl violet acetate . * $p < 0.05$; *** $p < 0.001$.

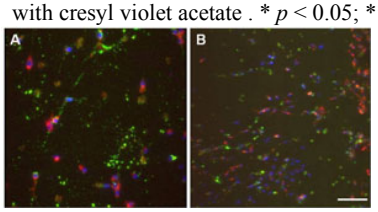

Fig. 2. Staining of collagen I (red), fibronectin (green) and DAPI (blue) from cell culture in PEG-peptide hydrogels: (A) 5% PQ at day 15 and (B) 10% PQ at day 21.Scale bar = 50 μm.

Micro-patterns of RGDS on PEGDA hydrogels by photolithographic patterning can confine the size, aspect ratio and orientation of single cells, which correlated to VIC activation shown by αSMA staining, while cells in the positive control sample with RGDS in a bulk condition were found to spread out randomly (Fig. 3).

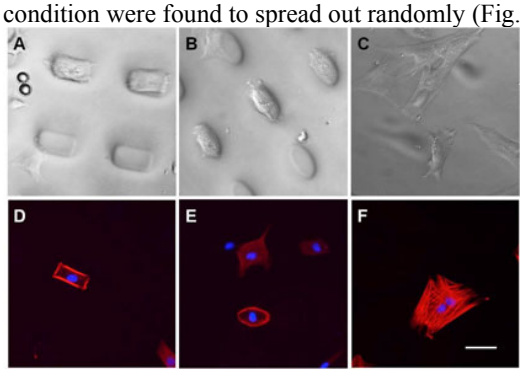

Fig. 3. Phase contrast images and fluorescent staining [αSMA (red) and DAPI (blue)] of VICs cultured on rectangle (A, D) and oval (B, E) patterns of RGDS as well as the positive control sample with RGDS in a bulk condition (C, F) in PEGDA hydrogels. Scale bar = 50 μm.

Conclusions: Laminate structures (stripe patterns) were created in PEGDA hydrogels by photolithographic patterning, which led to anisotropic mechanical behavior of the hydrogels, mimicking that of aortic valve leaflets. Cell adhesion and elongation, as well as production of new extracellular matrix proteins were influenced by the composition of bioactive peptides (cell-adhesion peptide RGDS and MMP-degradable peptide PQ) in PEGDA hydrogels. In addition, VIC activation was found to correlate to cell size and morphology, which can be modulated by photolithographic patterning of RGDS.

References: [1] Yacoub MH, Nat Clin Pract Cardiovasc Med. 2005; 2:60-1. [2] Cannegieter S, Circulation 1994; 89:635-41. [3] Fann JI, Ann Thorac Surg. 1996; 62(5):1301-1311.

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