Bioadhesive and Biodegradable Modification of Poly(ethylene glycol) Hydrogels for Modulating Cellular Responses

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Introduction: Poly(ethylene glycol) (PEG)-based hydrogels have been the primary choice of synthetic hydrogel materials for tissue engineering because they are biocompatible, non-immunogenic, resistant to protein adsorption, and easy for specific modification. PEG hydrogels typically exhibit minimal or no intrinsic biological activity. To incorporate bioactivity, short peptide sequences derived from native extracellular matrix (ECM) proteins, such as fibronectin and collagen, have been incorporated into PEG hydrogels to make biomimetic PEG hydrogels. Here, we report on a new strategy for ECM-mimetic modification of PEG hydrogels with incorporation of cell-adhesive and collagenase-sensitive peptide sequences for modulating cellular responses.

Methods: Diaminopropionic acid (Dap)-capped RGD peptide, Dap-GRGDSP and collagenase-sensitive peptide (CSP), GPQGIAGQ-Dap were synthesized on PAL resin by solid phase peptide synthesis (SPPS)[1]. RGD-PEGDA and CSP-PEGDA were synthesized by conjugating RGD and CSP peptides with acryloyl-PEG-NHS (Mw 3400), respectively (Figure 1). Hydrogel disks (diameter 8 mm, thickness 1 mm) were fabricated with 20% (w/v) of total macromers and 0.1% (w/v) of Irgacure 2959 in PBS under UV (365 nm) for 10 min. Human pulmonary artery endothelial cells (ECs) were seeded on the hydrogel surfaces, imaged on a phase contrast microscope and quantified by MTS assay[2].

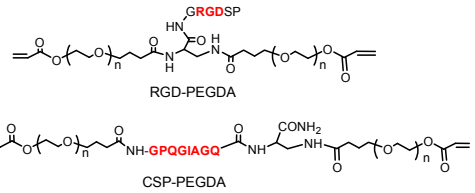

Figure 1. Structures of RGD-PEGDA and CSP-PEGDA

Results: Both biomimetic macromers, RGD-PEGDA and CSP-PEGDA can be photopolymerized to form hydrogels. Phase contrast images show that ECs seeded on PEGDA hydrogels assumed a rounded morphology with no evidence of spreading at both time points (Figure 2), suggesting that ECs have only weak, non-specific interactions with this material. ECs seeded on the RGD-PEGDA hydrogels showed higher initial cell attachment and cell spreading after 3 h. The enhanced cell attachment and spreading were attributed to the specific binding of RGD ligands to the integrin receptors on EC surfaces.

MTS assays show that all RGD-PEGDA (5-20%) hydrogels (with 15-0% PEGDA) had significantly higher ($p < 0.001$) EC population than PEGDA hydrogels 96 hrs after EC seeding (Figure 3A). Increasing RGD density resulted in higher EC proliferation due to more initial EC binding on the hydrogels surfaces.

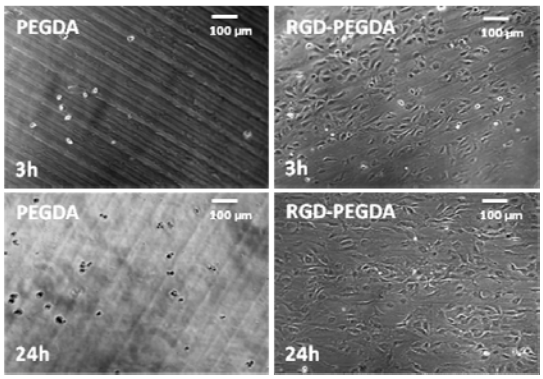

Figure 2. Phase contrast images observed at 24 hrs after EC seeding on PEGDA and RGD-PEGDA hydrogels.

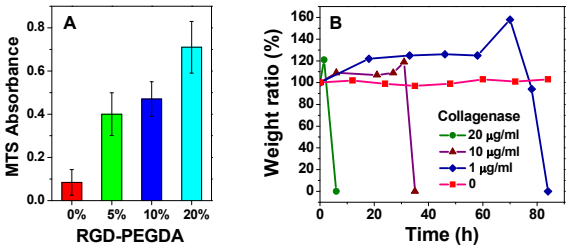

Figure 3. (A) Effect of RGD density on EC proliferation. (B) Degradation of CSP-PEGDA hydrogels in vitro

CSP-PEGDA hydrogels were incubated with collagenase with concentration of 1-20 μg/ml at 37°C. The results (Figure 3B) show that CSP-PEGDA hydrogels can be degraded at relatively low collagenase concentration (6 h with 20 μg/ml collagenase) and their degradation were dependent on the dose of collagenase.

Conclusions: RGD-PEGDA was synthesized with the attachment of the RGD peptide in the middle of the PEG chain, which is advantageous over RGD-PEG monoacrylate to form hydrogels with better control of peptide spatial organization and minimizing the affects of higher peptide incorporation on mechanical properties. CSP-PEGDA were synthesized with a collagen type I-derived collagenase-sensitive sequence, GPQGIAGQ in the middle of the PEG chain, which provides the resultant hydrogels with proteolytic degradation. Further study on the copolymerization of RGD-PEGDA and CSP-PEGDA is in progress, which will lead to the formation of bioactive PEG hydrogels with dual biofunctions, including specific cell adhesion and biodegradation.

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References:

1. Zhu J, et al. Macromolecules 2006; 39: 1035-1037.
2. Zhu J, et al. Bioconj. Chem. 2009; 20: 333-339.