Engineering a cell-adhesive and biodegradable hydrogel for smooth muscle cell attachment and growth

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Statement of Purpose: Intimal hyperplasia (IH) is one of the main impediments to successful implementation of small diameter vascular grafts. The overall goal of the project is to engineer a biomimetic scaffold system for regulation of SMC functions to minimize IH. To accomplish this, a biomimetic scaffold requires incorporation of mechanisms for cell attachment, biodegradation, and a drug delivery system for modulation of SMC functions. This report focuses on the cell attachment and biodegradation components of the biomimetic system. The design of the scaffold is based on poly (ethylene glycol) (PEG) system which is modified by mimicing the properties of extracellular matrix (ECM). GRGDSP cell-adhesive peptide derived from fibronectin, and GPQGIAGQ collagenase-sensitive peptide derived from collagen type I have been incorporated into the PEG chain. Copolymerization of biomimetic PEG macromers leads to the formation of bioactive PEG hydrogels with dual functionality, including cell adhesion and biodegradation.

Methods: The cell-adhesive peptide, GRGDSP and Diaminopropionic acid (Dap)-capped collagenase-sensitive peptide (CSP), GPQGIAGQ-Dap were synthesized by solid phase peptide synthesis (SPPS). RGD-PEGMA and CSP-PEGDA were synthesized by conjugating RGD and CSP peptides with acrylate-PEG-Succinimidyl Valerate (SVA) (Mw 3400), respectively. The synthesis of peptides and peptide conjugates were confirmed by matrix assisted laser deprotection/ionization mass spectroscopy (MALDI-MS). Hydrogel disks were fabricated from 10% (w/w) CSP-PEGDA hydrogel, ± 2% (w/w) RGD-PEGMA using 0.1% (w/v) of Irgacure 2959 in PBS under UV light (365 nm) for 10 minutes. The *in vitro* degradation behavior of hydrogels was studied by incubation of CSP-PEGDA hydrogels in collagenase solution (0-20 μg/mL) at 37°C. Cell attachment and growth was studied by seeding human coronary artery smooth muscle cells (HCASMC) on the hydrogel and fibronectin (FN) coated tissue culture plates (1 μg/cm^2), which were imaged with phase contrast microscopy and quantified by CytoTox 96 assay.

Results: MALDI-MS characterization showed successful synthesis of the biomimetic macromers, RGD-PEGMA and CSP-PEGDA, which were photopolymerized to form hydrogels. The *in vitro* degradation results showed that CSP-PEGDA hydrogels can be degraded at relatively low collagenase concentration (24 hours with 5 μg/mL collagenase), and the degradation time is decreased with the increase of collagenase concentration (4 hours with 20 μg/mL collagenase). Phase contrast images showed that there was little cell attachment and cell spreading on CSP-PEGDA hydrogel after seeding of SMCs for 24 hours, while SMCs seeded on CSP-PEGDA hydrogel with 2% RGD showed increased cell attachment and cell spreading (Figure 1). Quantification of cell number by CytoTox 96 assay (Figure 2) also showed that SMCs attached and grew well on the RGD bearing hydrogels, comparable with FN coated surfaces.

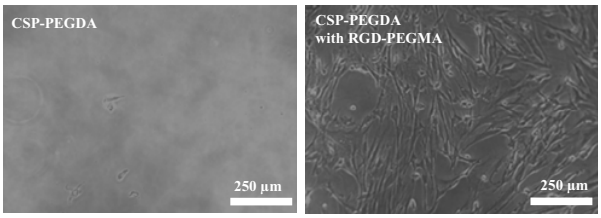

Figure 1. Phase contrast images observed at 24 hours after HCASMC seeding on CSP-PEGDA and RGD bearing CSP-PEGDA hydrogels

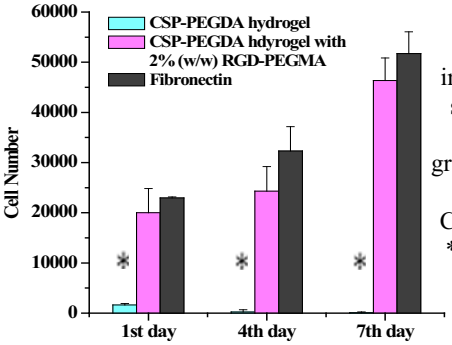

Figure 2. The influence of RGD sequence on the attachment and growth of SMCs on hydrogel by CytoTox 96 assay *: $p<0.05$ vs. FN coated surfaces

Conclusions: The incorporation of RGD-PEGMA into hydrogels showed enhanced cell attachment and spreading of SMCs on hydrogel surfaces. The enhancement can be attributed to the binding of RGD ligands to the integrin receptors on SMC surfaces. Collagenase-sensitive sequence, GPQGIAGQ in the middle of the PEG chain, provides the mechanism for collagenase-stimulated proteolytic degradation. Copolymerization of RGD-PEGMA and CSP-PEGDA results in the formation of cell-adhesive and biodegradable PEG hydrogels. Further study on the influence of cell-adhesive peptide and collagenase-sensitive peptide on the cell dependent degradation and 3-D smooth muscle cell growth and migration is in progress.

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References:

1. Beamish JA, Fu AY, Choi A, Haq NA, Marchant KK, Marchant RE. Biomaterials, 2009(30): 4127-4135.