Smooth Muscle Cell Migration in 3D Biomimetic Poly(ethylene glycol) Hydrogels

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Statement of Purpose: Smooth muscle cell (SMC) migration plays a key role in both physiological and pathological situations, ranging from vascular development to intimal hyperplasia. The goal of this project is to engineer a biomimetic scaffold as a model system for studies of smooth muscle cell migration in three dimensions. To accomplish this goal, poly (ethylene glycol) (PEG) based hydrogels were designed as the scaffold substrate and to mimic properties of the extracellular matrix (ECM), cell adhesive peptide (RGD) derived from fibronectin, and collagenase sensitive peptide (GPQGIAGQ) derived from collagen type I were incorporated into the PEG macromer chain. Copolymerization of biomimetic PEG macromers facilitates the formation of bioactive PEG hydrogels. By utilizing this biomimetic scaffold, the effect of RGD concentration, MMP sensitivity and gel matrix density on 3D SMC migration was investigated systematically.

Material and Methods: The cell adhesive peptide, GRGDSP (RGD) and diaminopropionic acid (Dap)-capped collagenase-sensitive peptide, GPQGIAGQ-Dap (GIA) were synthesized by solid phase peptide synthesis (SPPS). RGD-PEGMA and GIA-PEGDA were synthesized by conjugating RGD and GIA peptides with acryloyl-PEG-SVA (Mw 3400), respectively. RDG-PEGMA and IGA-PEGDA were prepared as negative controls. Hydrogel disks were fabricated with desired amount of macromers and Irgacure 2959 (0.1%, w/v) in PBS under UV light (365 nm) for 10 min. The *in vitro* degradation behavior of the hydrogels was studied by incubation of GIA-PEGDA hydrogels in collagenase solution over the concentration range of 0-1 μg/ml at 37°C. Cell attachment and growth was studied by seeding human coronary artery smooth muscle cells (HCASMCs) on the hydrogel and fibronectin (FN, 1 μg/cm^2) coated tissue culture plates, which were imaged with phase contrast microscopy and quantified by PicoGreen assay. Cell migration was studied by a modified 3D cell invasion experiment. Generally, HCASMCs entrapped in matrigel were polymerized within PEG hydrogels, then cell outgrowth was assessed microscopically.

Results: The *in vitro* degradation results (Fig. 1) showed that GIA-PEGDA hydrogels (10%, w/w) could be degraded in the collagenase solution. The degradation time decreased with increasing collagenase concentration. Quantification of cell number by PicoGreen assay (Fig. 2) showed that HCASMCs attached and grew on the hydrogels formed from GIA-PEGDA (10%, w/w) with 5 mM RGD, comparable with the FN coated surfaces. Cell attachment was limited on hydrogels with 5 mM RDG (Fig. 1b). The 3D Cell invasion experiments showed that HCASMCs migrated from the pellet into the 3D gels formed from GIA-PEGDA (5%, w/w) with 5 mM RGD, but cell migration was absent within IGA-PEGDA hydrogels (5%, w/w) (Fig. 3). The cell adhesive ligand (RGD) was also required to facilitate cell migration. The RGD ligand concentration mediated cell migration in a biphasic manner, with maximum cell migration observed at the concentration of 0.625 mM in 5% GIA-PEGDA hydrogels (Fig. 4). At constant RGD concentrations (0.625 mM), increasing GIA-PEGDA concentration from 5% to 7% decreased migration distances dramatically (Fig. 5).

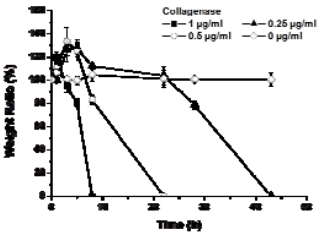

Fig. 1. *In vitro* degradation of GIA-PEGDA hydrogels (10%, w/w) in collagenase solution at 37 ˚C (0-1 μg/ml)

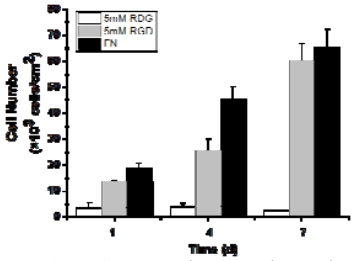

Fig. 2. SMC attachment and growth on GIA-PEGDA hydrogel (10%, w/w) with 5mM RGD or 5 mM RDG quantified by PicoGreen assay (Fibronectin serves as positive control)

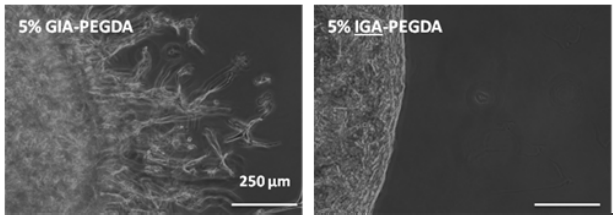

Fig. 3. SMC migration in 3D hydrogels formed from 5% GIA-PEGDA or 5% IGA-PEGDA with 5 mM RGD

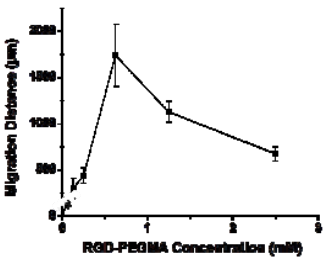

Fig. 4. The effect of RGD concentration (0-2.5 mM) on SMC migration in 3D GIA-PEGDA hydrogel (5%, w/w)

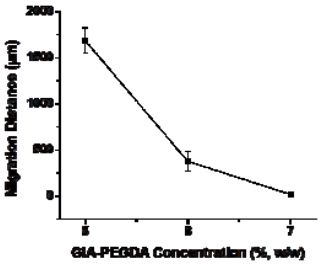

Fig. 5. The effect of GIA-PEGDA concentration (5-7%, w/w) on SMC migration in 3D hydrogel with 0.625 mM RGD

Conclusions: Copolymerization of RGD-PEGMA and GIA-PEGDA results in the formation of bioactive PEG hydrogels with cell adhesion and biodegradation properties. For cell migration to occur, both cell adhesive and proteolytically degradable peptide sequences were required in the hydrogels, and cell adhesive ligand mediated cell migration in a biphasic manner. Further, the gel matrix density of 3D matrices also played a key role in SMC migration in 3 dimensions.

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