Patterned Polyethylene glycol Coatings for Peptide Presentation and Cellular Adhesion

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Statement of Purpose: Biomaterials can mimic specific components of the native extracellular matrix (ECM) and provide a method for studing the cellular response. Cell adhesion, proliferation and differentiation are influenced by ECM signals. (Saha, K. Cur Opin Chem Bio. 2007;11: 381-387). Creation of a biomaterial environment where peptide concentrations can be controlled and quantified is advantageous for correlating stem cell behavior with presented peptide signals. Many typical two dimensional biomaterials require a specific substrate type, have limited stability in culture media, and the absolute peptide concentration may be difficult to determine.

Herein, we report the synthesis of a physically stable, substrate independent, polyethylene glycol (PEG) based coating that has specific functionality for crosslinking and peptide attachment. PEG was used because it provides a 'blank slate" background upon which adhesive peptide signals, (Arg-Gly-Asp-Ser-Pro) RGDSP specifically, may be introduced to promote cellular adhesion. An elastomeric stencil was used to pattern different concentrations of peptide into small circular regions on the coating. Patterning allows for combinatorial screening of different peptide densities and for isolation of smaller cell populations on the same substrate, eliminating well-to-well variations. The results detailed herein, highlight the RGDSP concentration dependence of cell adhesion, spreading and focal adhesion formation.

Methods: We synthesized a new amphiphilic PEG-based copolymer containing polyethylene glycol methyl ether methacrylate (PEGMEMA), polyethylene glycol methacrylate (PEGMA) and glycidyl methacrylate (GMA). The copolymer, P(PEGMEMA-*r*-PEGMA-*r*-GMA) was synthesized with a ratio of 85:10:5 by atom transfer radical polymerization. The copolymer was first spin-coated onto silicon wafers or glass slides to make a polymer coating (30 nm thickness) and then crosslinked for stability by thermal annealing. The coatings were modified with the peptide sequence GGGRGDSP and a scrambled sequence GGGRDGSP *via* 1,1'-carbonyldiimidazole coupling chemistry. Patterning of 1 mm in diameter circles was achieved using a polydimethylsiloxane (PDMS) mask. (Koepsel, J.T. J Mater Chem. 2012; 22: 19474-19481.) The patterned substrates were incubated with human mesenchymal stem cells (hMSCs) in α-MEM with 10% fetal bovine serum for 24 hours and then fixed and stained. Lastly, in a competitive binding experiment, cells were introduced to soluble cRGDfV (a high affinity cyclic RGD ligand) or the cRADfV control after 8 hours of standard culture.

Results: We have created a PEG based coating that is physically stable in aqueous solutions; meaning they persisted on the substrate over a period of 1 month. Coatings also swelled in water, increasing up to 80 percent of their original thickness. This interaction with water may improve accessibility of ligands and help facilitate ligand-receptor binding during initial cell attachment.

X-ray photoelectron spectroscopy (XPS) was utilized to quantify the amount of RGDSP on the coatings. The coating itself contained no nitrogen atoms; therefore nitrogen was a good elemental marker to quantify the amount of peptide. The coating had a concentration of 12.6 ± 6.14 pmol cm^{-2} RGDSP, calculated using known equations. (Paoprasert, P. J Mater Chem. 2010; 20: 2651-2658.) In addition, XPS mapping was used to visually determine the distribution of peptide on the surface and show small fluctuations of concentration over a square 2.2 mm area.

Coatings modified with scrambled peptide resisted adhesion and spreading of hMSCs, while coatings with RGDSP promoted cell adhesion. There was a notable difference in number of cells, cell area and the number of focal adhesions per cell on 10, 5, and 1 percent RGDSP conditions. Varying the ratio of RGDSP to scrambled peptide kept the total concentration of peptide on the surface the same. Further, we observed competitive detachment of hMSCs from coatings in cultures containing soluble cRGDfV. Competitive detachment demonstrated cell adhesion was specifically mediated by receptor binding to immobilized RGDSP on the surface.

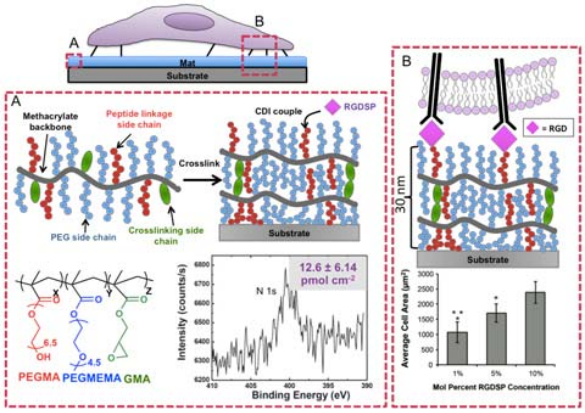

Figure 1. (A) A schematic and chemical structure of the copolymer P(PEGMEMA-*r*-PEGMA-*r*-GMA) and a XPS spectra of the nitrogen N 1s peak. (B) Schematic representation of the copolymer coating, showing hMSC adhesion to RGDSP. hMSCs average projected area increased as the concentration of RGDSP increased. A significant decrease, compared to the 10% condition is indicated by an asterisk, $p < 0.05$.

Conclusions: The PEG coatings discussed herein were used as a new material platform to study hMSC adhesion, spreading and focal adhesion formation. Coatings were resistant to hMSC adhesion when modified with scrambled peptide. The stability, patternability, ease of application of mats to a range of substrates and ability to precisely quantify bound peptides by XPS make them ideal for studying the long term presentation of ECM components to hMSCs.