Modulation of Cellular Adhesion by Changing Substrate Thickness and Adhesive Peptide Concentration

Derek M. Doroski, Brian H. Nguyen, Johnna S. Temenoff

Coulter Department of Biomedical Engineering, Georgia Institute of Technology and Emory University, Atlanta, GA, USA

Introduction: Tissue engineering approaches involving transplantation of tendon/ligament fibroblasts and/or marrow stromal progenitor cells (MSCs) have been proposed to improve regeneration of tendons and ligaments after injury. In our laboratory, a novel oligo(poly(ethylene glycol) fumarate) (OPF) biomaterial with variable fabrication and synthesis parameters is being explored as a cell carrier for these applications. Since the adhesion characteristics of the cell delivery vehicle can influence response of resident cells, this study examined the adhesion properties of two OPF formulations in response to changes in adhesion peptide concentration, biomaterial thickness, and swelling.

Methods: OPF with a poly(ethylene glycol) (PEG) chain of molecular weight 3kDa or 10kDa and a poly(ethylene glycol) diacylate (PEG-DA, nominal Mₙ 3,400) crosslinker, were combined in a 1:1 wt/wt ratio and suspended in saline (75 wt% liquid). For thick hydrogels only the OPF with a 3kDA PEG chain was used. Polymer solutions were cross-linked in thin (0.7 mm) or thick (3 mm) disc-shaped (14 mm radius) molds (10 min at 37 °C) using ammonium persulfate (APS) and N,N,N',N'-tetramethylethlenediamine (TEMED) thermal radial initiators (0.018 M). Thin hydrogels were fabricated with 0, 0.1, or 1 μmol RGD/g swollen hydrogel. Thick hydrogels were fabricated with 0, 1, or 3 μmol RGD/g swollen hydrogel. Ligament fibroblasts or MSCs were seeded (39,750 cells/cm²) on top of the thin or thick hydrogels in an area confined by stainless steel annuli (19 mm long, ID: 9.8 mm, OD: 15.5 mm). At 24 hours hydrogels were rinsed to remove non-adherent cells and at least four representative phase contrast images per sample were taken. For quantification, the number of cells per image was counted and cell numbers from multiple hydrogels in a particular group were averaged (n=4). These averages were divided by the area of the image to obtain the cellular density in cells/cm². Data from studies was analyzed for significance using ANOVA and Tukey’s Multiple Comparison Test (p ≤ 0.05).

Results: MSCs and fibroblasts demonstrated increasing adhesion with increasing concentrations of tethered RGD peptides (Fig. 1). However, the number of adherent cells was unaffected by either molecular weight of the OPF PEG chain, which can alter the hydrogel swelling ratio (data not shown), or the cell type used. For a given RGD concentration, fewer adherent cells were observed on thick (MSCs, 1 μmol RGD/g: 1067 ± 568 cells/cm²; Fibroblasts, 1 μmol RGD/g: 1474 ± 471 cells/cm²) than thin (MSCs, 1 μmol RGD/g: 29214 ± 4271 cells/cm²; Fibroblasts, 1 μmol RGD/g: 25332 ± 7579 cells/cm²) hydrogels. Additionally, statistically greater numbers of adherent fibroblasts were only found on thick hydrogels at an RGD concentration 3 times higher than that used with the thin hydrogels.

Phase contrast images revealed a round morphology in both MSCs and fibroblasts on thin and thick hydrogels without RGD (Fig. 3). Thin hydrogels with RGD promoted spreading of both MSCs and fibroblasts; however this change in morphology was not seen with thick hydrogels at any RGD concentration.

Conclusions: Both marrow stromal cells and fibroblasts demonstrated an ability to adhere to thin OPF hydrogels in response to RGD adhesion peptides, regardless of the length of the OPF PEG-chain. However, in thicker gels, only fibroblasts were able to adhere at higher RGD concentrations, and little cell spreading was observed. Although swelling properties were similar for both thin and thick gels for a given OPF type (data not shown), it is possible that the ligand density at the surface or the mechanical properties of the underlying gel were altered in the thicker constructs. Further studies are required to elucidate the complex interactions of mechanical properties and ligand availability on adhesion of both progenitor and committed cell types for ligament tissue engineering applications.

Acknowledgements: Georgia Tech/Emory Center for the Engineering of Living Tissues, Arthritis Foundation.