Quantitative Study of the Effects of PEG Substrate Physical Properties and Degradation Kinetics on Fibroblast Cell Migration

PI: Georgia Papavasiliou (BME); Co-PIs: Eric Brey (BME), Fouad Teymour (ChBE)
Graduate Student: Sonja Sokic (PhD Candidate, BME)

Project Description:
Engineering functional tissue replacements relies on a variety of inputs that must be provided to cells with spatial and temporal control in order to direct tissue development. These inputs include insoluble extracellular matrix (ECM) proteins, as well as ECM physical properties which are important for mediating cell adhesion, proliferation and migration. While naturally derived ECM materials have been used to study cell behavior they do not offer the versatility for investigating effects of specific protein components or the effects of substrate physical properties on cell response. As an alternative, synthetic hydrogels of polyethylene glycol diacrylate (PEGDA) have been widely used as scaffolds for tissue engineering applications since they offer an environment for controlled study of cell-substrate interactions.\(^1\) PEG hydrogels formed by free-radical photopolymerization in the presence of photoinitiators induce gel formation in the presence of visible or UV light. These biomaterials have been shown to regulate cell behavior due to their biocompatibility, their inherent capability for tuning material physical properties and their ability to selectively integrate key components of the native ECM into their crosslinked network. This is accomplished by incorporating adhesion sites, growth factors and proteolytically degradable sequences within PEG-DA hydrogels.\(^2\)

Although significant progress has been made with respect to exploring the effects of biospecific molecules on cell response on PEG hydrogels, the clinical success of these materials relies on several properties representative of the natural ECM such as the scaffold microarchitecture (crosslink density and mesh size), adhesion specificity, growth factor and degradation site availability which individually and collectively regulate cell behavior (adhesion, proliferation and migration).

Hydrogel formation is kinetically controlled and the final hydrogel properties are highly dependent on the polymerization conditions.\(^3\) A thorough quantitative study investigating the effect of polymerization conditions on scaffold material properties as well as on the level of incorporation of biologically functional molecules within PEGDA hydrogels has yet to be elucidated. This project coupled computational modeling and experimentation to address the following Specific Aims:

**Specific Aim 1.** Determine the effect of polymerization conditions on PEGDA hydrogel crosslink density and compositions of incorporated functional biomolecules.

**Specific Aim 2.** Quantify fibroblast cell (FC) behavior on degradable PEGDA scaffolds in response to variations in hydrogel crosslink density and compositions of incorporated functional biomolecules in vitro.

These studies will have a far-reaching impact on the field of tissue engineering and provide important insight on the effects of biophysical properties on FC migration which plays an important role in many processes such as wound healing and angiogenesis.

Results

**Specific Aim 1:**
1.1 We have developed a computational model of biofunctional PEGDA hydrogel formation based on the kinetics of free-radical photopolymerization that can be used as a guide to define conditions that result in hydrogels with properties that dictate cell behavior. The model accounts for the copolymerization of cell-specific adhesion site monoaacrylate macromers of RGD with diacrylate macromers of the crosslinking agent PEGDA and predicts the hydrogel crosslink density and the composition of pendant RGD peptides in the hydrogel. Model predictions compare favorably to experimental data. Figure 1 indicates that crosslink density increases with photopolymerization time (Figure 1). The model also predicts the incorporation of biofunctional moieties in the PEG backbone as conditions are varied. Model predictions agree with experimental data of RGD incorporation (obtained via radiolabeling experiments) for variations in initial concentrations of PEGDA, RGD and polymerization time as shown in Figures 2 and 3. Numerical simulations indicate that the model is capable of capturing dynamic features (similar to those observed experimentally) which may be difficult to obtain solely through experimentation (Figure 3). Future work will focus on including kinetic equations in order to quantify hydrogel biophysical properties in the presence of additional biofunctional components (growth factors and enzymatically sensitive degradation sites) in the hydrogel network. The model will require further validation with experimental data in the presence of these additional biofunctional factors. Computational models of biofunctional hydrogel formation have not been previously developed.

1.2. Swelling experiments were used to quantify the average molecular weight between crosslinks (a property inversely proportional to crosslink density) for a range of initial concentrations of the RGD adhesion macromer previously reported to promote cell adhesion on the surface of PEG hydrogels. Swelling data indicate that the hydrogel crosslink density is not significantly altered with variations in initial RGD concentration (0-10mg/mL) irrespective of the PEGDA macromer molecular weight (3400 and 8000 Da) (Figure 4). Further studies investigating a wider range of RGD concentrations are required to determine RGD precursor effects on crosslink density.
1.3. The natural process of cell migration requires degradation of the ECM via secretion of proteolytic enzymes by cells. To study fibroblast migration in 3D (within as opposed to on the surface of the hydrogel), PEGDA hydrogels containing RGD were modified to include a collagenase-sensitive peptide domain that would degrade with cell-secreted collagenase enzyme and therefore allow cell invasion and migration through the hydrogel. As an initial study, we looked at the degradation rates of hydrogels in the presence of collagenase enzyme solution. Complete degradation of the hydrogel resulted in ~7 days and was found to be dependent on enzyme concentration (Figure 5). As a further study of physical properties, we looked at the swelling behavior of degradable hydrogels (collagenase-sensitive) with varying ratios of degradable to non-degradable (PEGDA MW=8000 Da) macromer precursor percentages based on model predictions. Results show that varying the ratios of these macromers alters the gel crosslink density and could potentially give more control over hydrogel degradation rates. Furthermore, a step increase response in crosslink density was observed as a result of a decrease in the precursor ratio of degradable to non-degradable macromers (Figure 6). However, we found that these conditions did not result in fibroblast cell invasion. To further increase the mesh size of the hydrogels, collagenase-sensitive hydrogels were formed with addition of equal wt% unacrylated (PEG MW=8000). Swelling behavior was evaluated and results show that addition of equal wt% unacrylated to acrylated PEG causes a significant increase in the swelling ratio of the hydrogel (Figure 7). In addition, significant increases in swelling are observed with a lower percentage of the PEGDA crosslinking macromer. Cell invasion studies were investigated using this formulation; however, again cell invasion was not observed.
We are currently investigating the effects of NVP concentration to quantify cell migration and determine whether decreases in NVP result in a higher incorporation of RGD in the gel. Further studies are required examining a wider range of NVP concentration on cell adhesion and to identify a range of biophysical properties predicted in Aim 1 we identified a range of conditions to fabricate hydrogels and tested cell response first on the surface of the gel (2-D model) and secondly within the hydrogel (3D model). Negligible changes in cell adhesion on the gel surface were observed when the initial concentration of the RGD precursor was varied. We hypothesize that this is due to the fact that hydrogel crosslink density was not affected with alterations in initial RGD concentration (Figure 4). However, increases in the prepolymer concentration of the accelerator N-vinyl pyrrolidone (NVP) resulted in a decrease in fibroblast cell adhesion on the gel surface (Figure 8). This corresponds to an increase in the tensile modulus (proportional to crosslink density) as shown in Figure 9 suggesting that the cells adhered less on the more crosslinked hydrogel surfaces. Further studies are required examining a wider range of NVP concentration on cell adhesion and to determine whether decreases in NVP result in a higher incorporation of RGD in the gel. We are currently investigating the effects of NVP concentration to quantify cell migration on the hydrogel surface.

Specific Aim 2:
2.1. Based on the biophysical properties predicted in Aim 1 we identified a range of conditions to fabricate hydrogels and tested cell response first on the surface of the gel (2-D model) and secondly within the hydrogel (3D model). Negligible changes in cell adhesion on the gel surface were observed when the initial concentration of the RGD precursor was varied. We hypothesize that this is due to the fact that hydrogel crosslink density was not affected with alterations in initial RGD concentration (Figure 4). However, increases in the prepolymer concentration of the accelerator N-vinyl pyrrolidone (NVP) resulted in a decrease in fibroblast cell adhesion on the gel surface (Figure 8). This corresponds to an increase in the tensile modulus (proportional to crosslink density) as shown in Figure 9 suggesting that the cells adhered less on the more crosslinked hydrogel surfaces. Further studies are required examining a wider range of NVP concentration on cell adhesion and to determine whether decreases in NVP result in a higher incorporation of RGD in the gel. We are currently investigating the effects of NVP concentration to quantify cell migration on the hydrogel surface.

2.2. In an effort to mimic the natural process of migration, 3D fibroblast migration was studied. This was accomplished by encapsulating spheroids (10,000 cells/spheroid) formed with carboxymethylcellulose (Sigma Aldrich, St. Louis, MO) within the precursor. Invasion of the spheroid within the hydrogel was measured by subtracting the area of
the spheroid at day 0 from the subsequent area occupied by the cells over time. Since cell invasion was not observed for the case of a hydrogel conjugated with a single enzymatic degradation site between the PEG crosslinks regardless of the PEG macromer molecular weight (3400 and 8000 Da), we further modified the hydrogel network to include multiple degradation sites within the PEG backbone also resulting in a further increase in the gel mesh size (PEGDA MW=15,700 Da). Cell studies were conducted using the higher MW modified PEGDA resulting in fibroblast invasion within 3-5 days as compared to non-degradable hydrogel controls (Figure 10). Individual fibroblasts were also encapsulated within these same hydrogels and monitored over time. Cells assumed a different morphology within 3-5 days with extended processes and formed cell-cell connections (Figure 10). In both cases (invading aggregate and cells suspended in degradable gels) hydrogels were formed by suspending 20ng/mL of basic fibroblast growth factor (FGF-1) with 5U/mL heparin the prepolymer solution. Future studies will focus on determining whether cell invasion is FGF-1 dependent. Additional studies will look at the effects of soluble FGF-1 (encapsulated in the gel) versus conjugated FGF-1 (FGF-1 covalently attached to the PEG backbone) on fibroblast invasion and migration.
Figure 10. Phase contrast and fluorescence images of fibroblast cells within PEGDA hydrogels. (A)(B)(C)(D)(E) are phase contrast images. (E)(G) are phalloidin stained fluorescence images. Fibroblast cell spheroids at day 7 are shown within (A) non-degradable PEGDA 8000 hydrogels, (B) collagen gel controls, (C) collagenase-sensitive (degradable) hydrogels , (D) image (C) zoomed 20X. (F) Single fibroblast cells suspended within degradable hydrogels on day 5. Phalloidin stained fluorescence images of (E) fibroblast cell spheroid within degradable hydrogel and (G) individual fibroblast cells within degradable hydrogel.

References

