

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: This project couples computational modeling and experimentation to investigate the effects of polymerization conditions on poly(ethylene glycol) diacrylate (PEGDA) hydrogel 1) physical properties and 2) incorporated levels of key components of native extracellular matrix for controlling fibroblast cell behavior (adhesion and migration) on and within scaffolds *in vitro*. The model is based on a free-radical photopolymerization kinetic mechanism where crosslinking reactions of PEG diacrylate with PEG monoacrylate biofunctional macromers result in hydrogel formation. These studies will provide significant insight on the effects of biophysical properties on fibroblast cell migration which play an important role in processes such as wound healing.

Results: 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 monoacrylate macromers of RGD with diacrylate macromers of the crosslinking agent PEGDA and predicts crosslink density and the composition of pendant RGD peptides in PEG hydrogels. Model predictions and experimental data indicate that crosslink density increases with photopolymerization time (Figure 1). We have found that model predictions compare favorably to experimental data of RGD incorporation (obtained via radiolabeling experiments) for varying conditions (initial concentrations of PEGDA, RGD and polymerization time) as shown in Figures 2 and 3. Simulations indicate that the model captures similar dynamic features to those

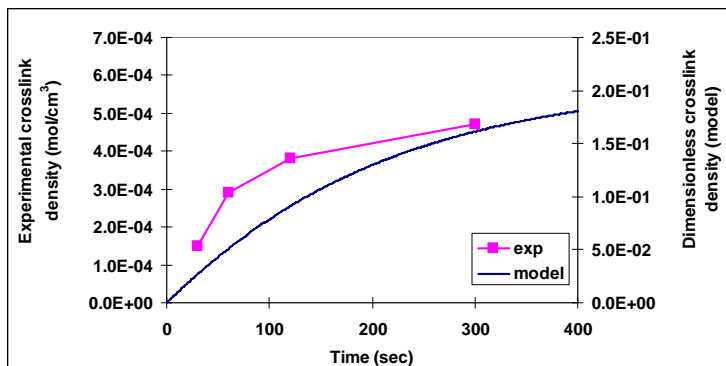


Figure 1. Effect of photopolymerization time] on hydrogel crosslink density. (n=3)

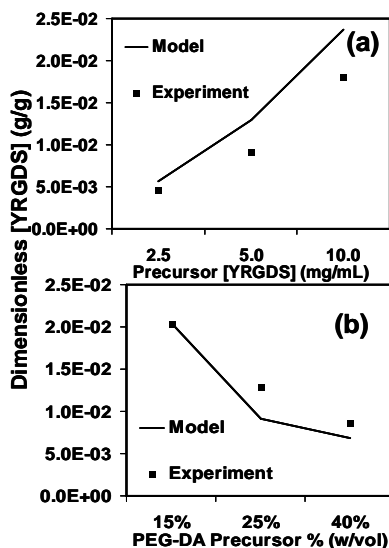


Figure 2. Effect of (a) initial [YRGDS] and (b) initial PEG-DA % on YRGDS incorporation in hydrogels (n=3).

observed experimentally which may be difficult to obtain solely through experimentation (Figure 3). For hydrogels engineered with RGD adhesion sites, the level of RGD incorporation in hydrogels will determine fibroblast cell adhesion and migration on the gel surface and within the hydrogel which is currently under investigation.

The natural process of cell migration requires cell secretion of proteolytic enzymes that degrade the extracellular matrix allowing cells to migrate into their underlying basement membrane. However, previous studies have indicated that the mesh size of PEG hydrogels is too small to allow for cell migration within PEG hydrogels.¹ To mimic cell migration in 3D, PEG hydrogels were engineered with degradable (collagenase-sensitive) peptide macromer domains that were crosslinked within the polymeric backbone

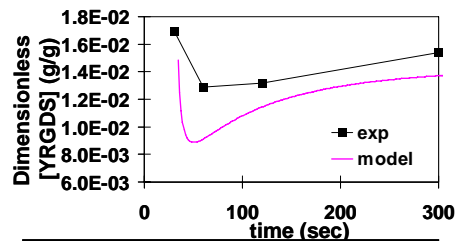


Figure 3. Final hydrogel [RGD] as a function of visible light exposure time.

with the goal that cell-secreted collagenase will result in degradation of these crosslinks enabling cell migration through

hydrogels. Preliminary studies of degradable hydrogels incubated in a collagenase enzyme solution show complete hydrogel degradation for gels polymerized only with a degradable crosslinker and that the gel degradation rate is a function of the enzyme concentration (Figure 4). Experiments were also conducted whereby the ratio of degradable (collagenase-sensitive) to non-degradable (PEGDA) macromer precursor percentages were varied at fixed macromer molecular weights (MW=8000 Da). Our results indicate that varying precursor ratios of degradable to non-degradable macromers alters gel crosslink density potentially allowing for control in gel degradation rates (Figure 5). Results in Figure 5 also indicate that a decrease in the precursor ratio of degradable to non-degradable (PEGDA) macromers causes a step increase in crosslink density. With the addition of RGD as the bioactive peptide we are currently investigating effects of the degradable to non-degradable precursor ratio on hydrogel crosslink density, rate of degradation, and fibroblast migration through the hydrogel.

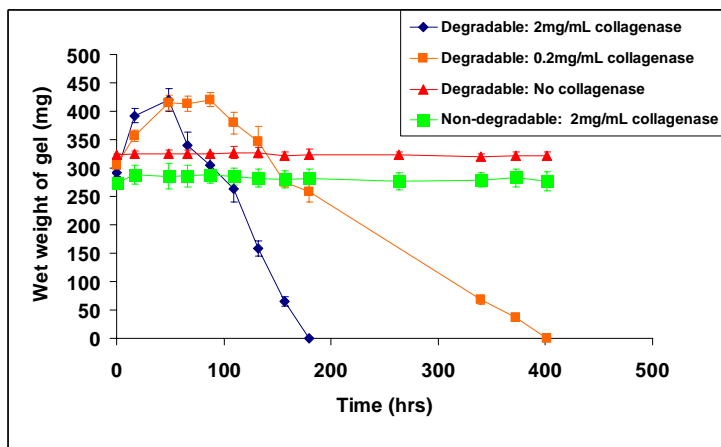


Figure 4. PEG hydrogels engineered with and without collagenase-sensitive (degradable) crosslinks. Hydrogels containing collagenase-sensitive crosslinks degrade in the presence of collagenase enzyme solution (n=3).

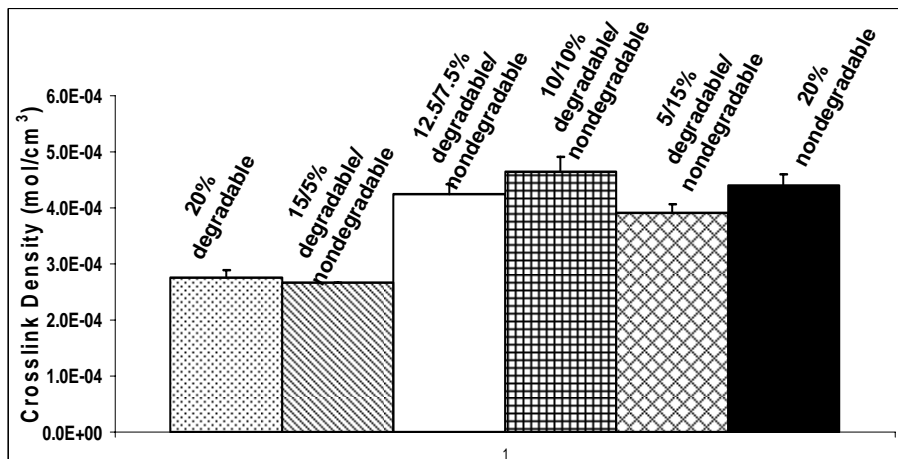


Figure 5. Effect of degradable to non-degradable macromer precursor ratio on hydrogel crosslink density. (n= 3)

Plans and Future Goals:

Aim 1). Determine the effect of polymerization conditions on PEGDA hydrogel crosslink density and composition of incorporated functional biomolecules.

- While we have developed the model and performed computational and experimental sensitivity analyses further studies are required for its validation. The model will be validated by investigating the effects of the following polymerization conditions (Initiator, co-initiator, RGD macromer and degradable macromer precursor concentrations, PEG macromer molecular weight and laser flux) on 1) hydrogel crosslink density and 2) YRGD incorporation. We have completed all experimental studies with respect to these variables for non-degradable hydrogels and quantified crosslink density and RGD incorporation as a function of these variables experimentally. Crosslinking of hydrogels via free-radical photopolymerization involve termination

reactions that result in the combination of large polymer radicals. These reactions become diffusion controlled at high macromer conversions leading to increases in the free-radical population and thus an acceleration in the rate of polymerization. Therefore, we are currently investigating the effects of diffusion controlled termination (gel effect) using the model. Once the “gel effect” is implemented model simulations will be compared to experimental data. Finally the crosslink density obtained experimentally is based on the Flory-Rehner equation³ while the model predicts a dimensionless crosslink density. We are currently developing methodologies for direct comparison of crosslink density between model and experiment.

- We have verified that the incorporation of enzymatically degradable domains in PEGDA hydrogels result in the formation of hydrogels that degrade in the presence of the enzyme and that adjustments in the ratio of degradable to non-degradable domains lead to changes in hydrogel mechanical properties. We are currently conducting studies to quantify the effect of this ratio on the kinetics of gel degradation. Future computational and experimental studies will also include the determination of gel crosslink density and incorporation efficiencies in the presence of both RGD and degradable domains in hydrogels for model validation.

Aim 2). Quantify fibroblast cell behavior on PEG-DA scaffolds in response to variations in hydrogel crosslink density and compositions of incorporated functional biomolecules in vitro.

- We are currently conducting fibroblast cell studies on non-degradable hydrogels based on crosslink density and RGD incorporation experimental data to quantify cell behavior on the hydrogel surface as described.
- We have conducted preliminary studies of fibroblast cell encapsulation within PEG hydrogels containing degradable crosslinks and RGD, however, there was no cell migration or gel degradation observed within the hydrogel in 3D. Further adjustment in gel mechanical properties will be required to optimize conditions for 3D cell migration based on the experimental and computational studies resulting from Aim 1. We have also modified the research design methods described in sub-Aim 2.2 to investigate the effects of polymerization conditions on 3D fibroblast migration by utilizing a published cell culture protocol which has previously shown success of cell invasion with PEG hydrogels in 3D.² As an alternative, fibrin clotted fibroblast cells will be encapsulated in gels to allow for degradation and cell migration in 3D. We anticipate that the addition of fibrin may accelerate the growth of cells and cell-secreted enzymes to enable gel degradation and cell migration.

References

1. Raeber GP, Lutolf MP, Hubbell JA. Molecularly engineered PEG hydrogels: A novel model system for proteolytically mediated cell migration. *Biophysical Journal* 2005, 89 (2): 1374-1388.
2. Pratt AB, Weber FE, Schmoekel HG, Müller R, and Hubbell JA. Synthetic extracellular matrices for in situ tissue engineering. *Biotechnology and Bioengineering*, 2004, 8(1): 27-36.
3. Peppas NA, Hilt JZ, Khademhosseini A, Langer R. Hydrogels in Medicine and Biology: From Molecular Principles to Biotechnology. *Advanced Materials* 2006, 18 (1345-1360).