# Mutli-Arm PEG Hydrogels Containing Collagen Sequence and Cell-Adhesive Sequence Support Enzyme Mediated Degradation and Endothelial Cell Proliferation

Derek R. Jones[1], Junmin Zhu[1], Roger E. Marchant[1]
[1]Department of Biomedical Engineering, Case Western Reserve University, Cleveland, OH 44106, USA

**Statement of Purpose:** Here we report on a new strategy for the incorporation of fibronectin derived cell-adhesive peptide and collagenase sensitive peptide to PEG hydrogels for modulating degradation, cell attachment, and proliferation. Functionalized hydrogels can be prepared in a single step by mixing proportions of thiol-containing peptide with 4-arm PEG acrylate, allowing for thiol-acrylate Michael addition to occur. This method is unique in that it combines peptide conjugation and polymerization into a single step, and removes the need for UV-mediated polymerization. Poly(etheyle glycol) (PEG)-based hydrogels have been used for tissue engineering because they are resistant to protein adsorption and are easily modified. Incorporation of ligands in the PEG network allows for control of cell-scaffold interactions.

**Methods:** Hydrogels were synthesized combining 4-arm PEG (MW = 10500) and collagenase sensitive peptide CGPQGIAGQC (CSP) in a 2:1 ratio at pH 8 for 1 hour.
The efficiency of the thiol-acrylate Michael addition was determined from an Ellman's assay using linear PEGDA (MW=575) and CSP, as well as the bi-functional peptide CGPQGIAGQCGRGDSP (RGD-CSP). The amount of unreacted thiol was calculated from the absorbance on a spectrophotometer with a wavelength of 410nm at 0 and 1 hour after the reaction.
To measure degradation, 10% (w/v) hydrogels were synthesized as described above. Upon polymerization, the hydrogel disks were incubated in Hanks Buffered Saline Solution overnight. Hydrogels were then placed in collagenase solutions at concentrations 1.0, 0.5, 0.25, and 0 µg/ml. At various time points, wet weights of the hydrogels were taken.
Thin film 5% (w/v) hydrogels for cell studies were synthesized onto coverslips. Cell adhesive hydrogels were synthesized by replacing 1.3 and 2.7 mM of the collagenase sensitive peptide with RGD-CSP. The non-cell adhesive negative control hydrogels were synthesized without the RGD-CSP peptide, and 1.0 µg/cm$^2$ fibronectin surface served as the positive control. HUVECs were seeded on all surfaces at 15,000 cells/cm$^2$ in serum free media. After 6 hours, media was changed to serum containing endothelial growth media. Phase contrast microscope images (10x) were taken at 1, 4, and 7 days, and a Picogreen assay was used to assess cell growth at these same timepoints.

**Results/Discussion:** Ellman's assay results suggest that after 1 hour, 98% of the thiols in CSP had reacted, and 96% of the thiols in RGD-CSP had reacted. This suggests that an incubation time of 1 hour is sufficient to incorporate both peptides into the backbone of the hydrogel construct.

In the degradation study, the time it took the hydrogels to degrade increased corresponding to a decrease in collagenase concentration. The negative control hydrogel that was incubated in 0 µg/ml collagenase solution underwent no significant change in mass.
For the cell attachment and proliferation study, the surfaces containing 1.3 and 2.7 mM RGD-CSP showed a low level of attachment when compared to the fibronectin surface, but higher level of attachment than the negative control surface. Both experimental surfaces had an increase in cell number over the 7 day period. The fibronectin surface became confluent by day 4, and any cells present on the CSP hydrogel with no RGD had detached by day 7.

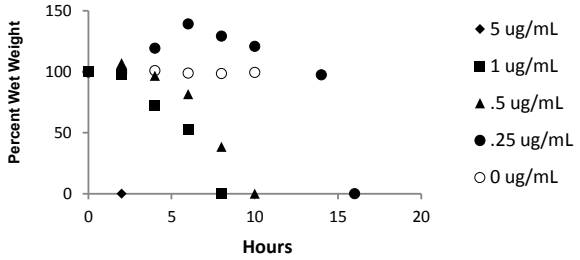


**Figure 1.** Collagenase mediated degradation of PEG hydrogels containing CSP over time.

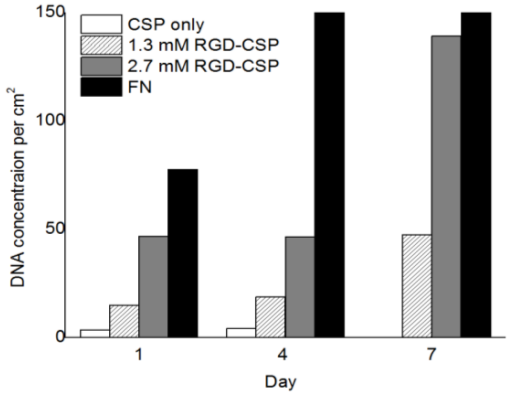


**Figure 2.** DNA concentration measured by Picogreen assay to assess cell attachment to PEG hydrogels containing variable concentrations of RGD-CSP for 1, 4, and 7 days.

**Conclusions:** These data suggest that synthesis of functionalized hydrogels can be formed using the one step thiol-acrylate Michael addition method, without the use of UV. Incorporation of the CSP into hydrogels allowed for enzyme mediated degradation and incorporation of the RGD-CSP sequence into the backbone of the polymer allowed for both cell attachment and proliferation.

**References:** Zhu JM., et al. Macromolecules. 2006;39:1305-1307.
**Acknowledgements:** The project described was supported by Grant Number RC1EB010795 from the National Institute of Health. The content is solely the responsibility of the authors and does not necessarily represent the official views of the National Institute of Health.