Hydrogels formed from RGD-containing PEG diacrylate support smooth muscle cell viability

Jeffrey A. Beamish[1], Junmin Zhu[1], Kandice Kottke-Marchant[2], Roger E. Marchant[1]
[1]Department of Biomedical Engineering, Case Western Reserve University, Cleveland, OH 44106, USA
[2]Department of Clinical Pathology, Cleveland Clinic Foundation, Cleveland, OH 44195, USA

Statement of Purpose: Poly(ethylene glycol) diacrylate (PEGDA) based hydrogels have attracted interest for tissue engineering applications. The poly(ethylene glycol) (PEG) backbone of the network is inherently resistant to protein adsorption, providing a scaffold for the incorporation of ligands that dictates cell-scaffold interactions. To date, these interactions have been mediated predominately by acryloyl-PEG-peptide conjugates tethered to a core PEGDA network. The addition of these components affects the physical properties of the gel. Using an analog of acryloyl-PEG-peptide conjugates, we have assessed the effect of incorporating this material on the gel network. We have also developed a diacrylate monomer which itself contains a peptide ligand, allowing for control of peptide density without altering the gel network. We have tested the ability of this material to support the attachment and proliferation of human vascular smooth muscle cells (SMCs) in 2D and SMC viability in 3D.

Methods: To investigate the effect of PEG monoacrylate (PEGMA) incorporation into the gel network, PEG monomethyl ether (MW=5000) was conjugated with acryloyl chloride to give an analog to acryloyl-PEG-peptides. This material (0-32 mM) was crosslinked into PEGDA (6K-PEGDA, MW=6000) hydrogels as previously described[1]. Gels were allowed to equilibrate in PBS overnight, and the mass swelling ratio (q) was determined. These values were compared with swelling predicted by the Peppas-Merrill model[2].

A diacrylate macromer that directly incorporates an RGD adhesive ligand (RGD-PEGDA) was synthesized as previously described[1]. Hydrogel disks were formed using 0.2 µm filtered 20 wt% RGD-PEGDA or 6K-PEGDA. For bulk seeding studies, hydrogels included 2×10^6 SMCs/ml. At each time point, one gel of each composition was stained using the LIVE/DEAD® viability assay and optical sections of the gel were imaged using a confocal microscope. The fraction of live and dead cells was quantified from these images and used to calculate the viable cell fraction in each gel. For surface seeding studies, hydrogel disks were inverted, equilibrated in PBS for 1 h, and seeded with SMCs. The gel surfaces were monitored with phase contrast microscopy over the duration of the study.

Results/Discussion: The addition of PEGMA to 10 wt% PEGDA hydrogels markedly reduced the mass swelling ratio, while it had little impact on 20 wt% PEGDA gels (Figure 1A). Interestingly for the 10 wt% PEGDA gels, the decrease in swelling exceeded the prediction of the Peppas-Merrill swelling model, suggesting that additional acryloyl moieties actually enhanced the network crosslinking. While the higher molar concentrations of PEGMA used here exceeded those typically used in tissue engineering studies, these data indicate that the addition of small amounts of PEGMA to loosely crosslinked PEGDA gels can alter the gel properties.

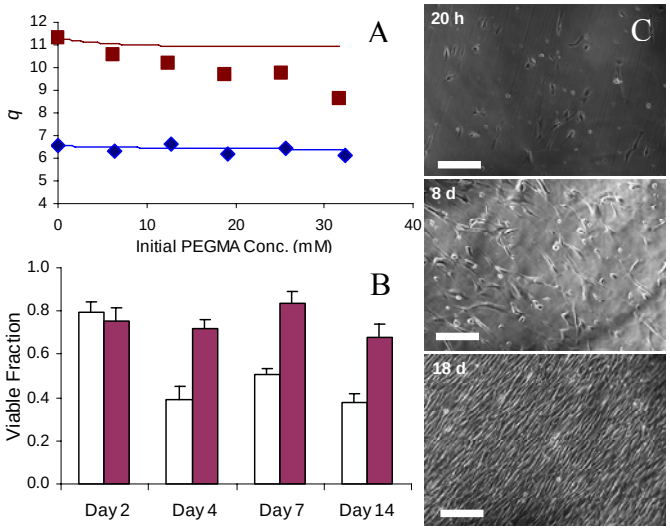

Figure 1. A. The effect PEGMA incorporation on hydrogel mass swelling ratio, q. (■: 10 wt% PEGDA, ◆: 20 wt% PEGDA; solid lines represent swelling predicted by Peppas-Merrill model assuming an identical elastic network to the [PEGMA] = 0 mM case). **B.** The fraction of viable SMC encapsulated in RGD-PEGDA (solid bars) versus PEGDA6k (open bars) over a two week culture period. Error bars represent standard deviation among image samples. **C.** Phase contrast micrographs of SMCs seeded on the surface of RGD-PEGDA hydrogels after 20 h, 8 days and 18 days. (Scale bar = 200 µm)

SMCs encapsulated in RGD-PEGDA maintained a high viable fraction over a 2 week culture period, while cells seeded in 6K-PEGDA showed decreased viability beyond 4 days (Figure 1B). SMCs seeded on the surface of RGD-PEGDA showed initial attachment and spreading after 20 hours (Figure 1C), while those on 6K-PEGDA did not attach. Furthermore, the RGD-PEGDA surface was capable of supporting proliferation and long-term culture up to 18 days, although this process occurred more slowly than on a tissue culture plate control. These results suggest that this material can support cell viability in 2D and 3D, and therefore is an excellent candidate for tissue engineering applications.

Conclusions: These data suggest that incorporation of monoacrylated ligands into PEGDA hydrogels can affect the physical properties of the hydrogel, especially for loosely crosslinked gels. The diacrylated RGD-PEGDA molecule, which can theoretically avoid these problems, can support cell attachment and proliferation on its surface and can support long term viability of encapsulated cells. Future work will continue to explore the physical properties of RGD-PEGDA hydrogels and to utilize this system for tissue engineering applications.

References:

1. Zhu JM. Macromolecules. 2006;39:1305-1307.
2. Peppas NA. J. Polym. Sci. Polym. Chem. 1976;14:441.