PEG HYDROGELS AS NERVE GUIDANCE MATERIALS

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INTRODUCTION

Nerve guidance conduits (NGCs) are currently under investigation to aid regeneration following injury to a nerve that results in a large defect. In engineering a system to improve regeneration of peripheral nerves, it may be important to provide proper signaling mechanisms to both the neurites and the Schwann cells. Bioactive polymers have been emerging as potential scaffolds that provide both support and signaling for cells, stimulating growth for tissue engineering applications. Various aspects of cell behavior may be influenced by such signals, including adhesion, proliferation, migration, and extracellular matrix synthesis. Polyethylene glycol (PEG) is one polymer that may be used as a scaffold, yet also rendered bioactive. PEG can be photopolymerized under mild conditions in the presence of cells to create a hydrogel that is biocompatible and non-toxic [1]. Bioactive molecules, such as cell adhesion ligands, growth factors, and proteolytic degradation sites, have previously been incorporated into PEG hydrogels and shown to influence adhesion, proliferation, migration, and extracellular matrix production of vascular smooth muscle cells [2,3].

For peripheral nerve regeneration, it will be important to determine which bioactive molecules need to be incorporated into the PEG hydrogels in order to improve neurite extension and Schwann cell migration, and subsequent regeneration of the nerve. As nerve cells secrete plasmin during neurite extension, the proteolytically degradable sequence NRV, which is targeted by plasmin, may be incorporated into the backbone of the PEG, rendering it biodegradable [4]. Further, this links the degradation of the scaffold to a biologic process, extension of the neurite. Alternatively, a PEG scaffold incorporating a sequence degradable by MMP-2, which is secreted by Schwann cells. Cell adhesion ligands will also need to be incorporated to provide attachment sites for the extending neurite and Schwann cells to the scaffold. The cell adhesion ligands RGDS, IKVAV, and YIGSR have all been shown to influence neurite extension. Additionally, a growth factor, such as nerve growth factor (NGF), could be incorporated into the PEG hydrogel. In the current study, cell adhesion ligands were incorporated into PEG hydrogels to examine their influence on neurite extension. Proteolytically degradable PEG hydrogels to be targeted by Schwann cells were also created and examined.

MATERIALS & METHODS

Neurite extension on PEG hydrogels

The rat pheochromocytoma cell line PC12 was used in the current study. Cell adhesion ligands examined were RGDS, YIGSR and the IKVAV-containing peptide CSRARKQAASIKVAVSADR (designated IKVAV). RGES was used as a non-adhesive control. Peptides were tethered to PEG by reacting the peptide with acryloyl-PEG_{3400}-NHS. Peptide-containing hydrogels were prepared by combining PEG_{6000} diacrylate, peptide-PEG-monoacrylate, and 2,2-dimethyl,2-phenylacetophenone in N-vinylpyrrolidone (450 ppm) in HEPES-buffered saline (HBS), placing the solution in a rectangular glass mold (0.5mm thick), and exposing to UV light (365 nm, 10 mW/cm^2) for 30 sec. In this manner, a three-dimensional crosslinked hydrogel was formed with the peptide covalently incorporated into the hydrogel. Discs (12mm diameter) were punched from the hydrogel and placed in 24-well plates with media for 24 hr at 37 ^oC. PC12 cells, in NGF-containing media (50 ng/ml) were then seeded on top of the hydrogels. After 72 hr, the hydrogels were rinsed gently with phosphate buffered saline, imaged, and the number of cells extending neurites was determined. Peptide concentrations between 0.2 and 4 μmol/ml were used. The effects of individual peptides and a combination of RGDS and YIGSR were examined.

Degradation of PEG hydrogels

An ABA block copolymer of PEG (A) and the peptide sequence GGPQGIASQGGK (B) was created by reacting the peptide with acryloyl-PEG_{3400}-NHS. Hydrogels were created using the block copolymer as described above, except that discs were created that were xxmm in diameter and 2mm thick. Gels were allowed to swell for 24hr in HBS. The gels were then placed in HBS with or without collagenase (0.2 or 2.0 mg/ml) in a 37^oC oven, and the mass of the gels was tracked over 29 days. Control hydrogels made with PEG_{6000} diacrylate were also placed in HBS with 2.0 mg/ml collagenase.

PC12 cells were able to adhere to and extend neurites on PEG hydrogels containing the adhesion ligands, but not on hydrogels with the control peptide RGES. This indicates that the adhesion and neurite extension was due to the presence of the adhesion ligand. Figure 1 shows PC12 cells extending neurites on a PEG hydrogel with RGDS covalently incorporated. RGDS, IKVAV, and YIGSR have also previously been found to promote neurite extension from PC12 cells embedded within agarose gels [5]. The effect of ligand density on neurite extension was examined using RGDS. As shown in Figure 2, an increase in RGDS incorporated in the hydrogel resulted in an increase in cells extending neurites. This was accompanied by a concomitant increase in the number of adherent cells.

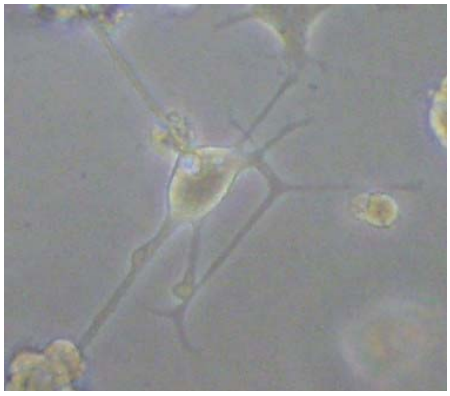

Figure1. PC12 cells extending neurites on a PEG hydrogel with RGDS covalently incorporated.

Also shown in Figure 2, more cells were able to extend neurites on RGDS-containing hydrogels than on YIGSR- or IKVAV-containing hydrogels when the peptides were incorporated at the same concentration. It is possible that different adhesion ligands will have different optimal concentrations for neurite extension, and this will be examined in future studies.

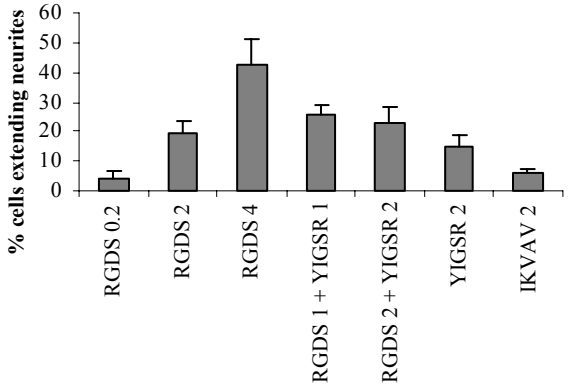

Figure 2. PC12 neurite extension on PEG hydrogels with covalently attached cell adhesion ligands (in µmol/ml).

Further, the effect of a combination of RGDS and YIGSR was examined. The two peptides were incorporated in equimolar concentrations such that the total peptide incorporated was either 2 or 4 µmol/ml. With 2 µmol/ml total peptide, there was more neurite extension for the combination of peptides than for either peptide alone. However, doubling the total peptide concentration for the combination of peptides resulted in a slight decrease in neurite extension, rather than the increase observed with RGDS alone. This may indicate that not only the specific combination of peptides but the amount of each peptide used can affect neurite extension. Further studies will examine various combinations of the adhesion ligands.

Hydrogel degradation

Hydrogels were created using a PEG diacrylate derivative with a proteolytically degradable peptide (GGPQGIASQGGK) in the backbone. This peptide sequence is degradable by a variety of matrix metalloproteases, including MMP-2, secreted by Schwann cells. Hydrogels containing the target peptide degraded in solutions containing collagenase but not in buffer alone (Figure 3). Further, control hydrogels that did not contain the target peptide did not degrade in solutions containing collagenase. These results indicate that a PEG scaffold can be created that will link degradation of the scaffold to protease secretion by cells.

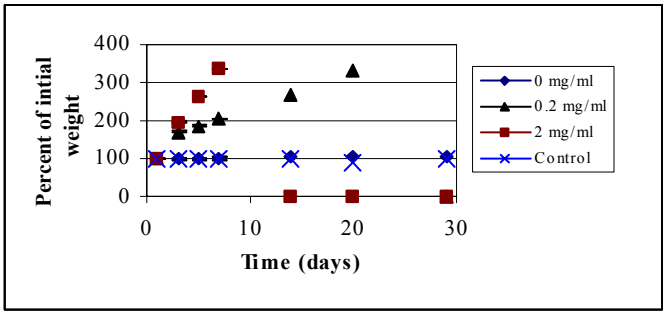

Figure 3. Degradation of PEG copolymers with collagenase.

The current study has shown that cell adhesion ligands covalently incorporated into PEG hydrogels allow PC12 cells to adhere to the hydrogels and extend neurites on the hydrogels. We have also shown that PEG hydrogels can be rendered susceptible to proteolytic degradation. Previous work has shown that growth factors can also be incorporated into PEG hydrogels [2]. Incorporating various cell signaling molecules (adhesion ligands, growth factors, proteolytic degradation sites) into a single PEG hydrogel may lead to a bioactive scaffold that can be used as a nerve guidance conduit for peripheral nerve regeneration.

ACKNOWLEDGMENTS

The authors would like to thank the Fletcher Jones Foundation for its support.

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