Orthogonal Photo-reactive Hydrogel of Tunable Stiffness for Guided Neurite Growth

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Introduction

Axon pathfinding is significantly influenced by growth substrate stiffness during development. For *in vitro* neural growth models, substrate stiffness is often used as a governing factor of neurite guidance, as it has a significant impact on the physiological response of projected neurites. Thus, substrates with tunable stiffness provide a valuable parameter for controlling neurite growth and guidance. Here we present a hydrogel substrate of polyethylene glycol (PEG) that independently polymerizes in the presence of visible light and degrades in the presence of UV light. We used a digital micromirror device (DMD) to pattern both visible and UV light, thereby spatially controlling the stiffness of the hydrogel. This approach allowed for the gel stiffness to be dynamically tuned with varying light irradiation times in order to direct neurite growth by mechanical cues alone.

Methods

Orthogonally photo-reactive PEG gels were composed of a UV-degradable PEG crosslinker (PEGdiPDA) [1], PEG acrylate (PEGA), PEG diacrylate (PEGDA), the visible light photoinitiator system eosin Y/ 1-vinyl-2-pyrrolidinone/ triethanolamine [2], and the free-radical scavenger TEMPO. The photosensitizer eosin Y excites near λ = 510 nm light and PEGdiPDA degrades in the presence of λ = 365 nm light. Laminin was added to the prepolymerization solution to enhance the cell adhesion character of the inert PEG gels. The PEG gels were polymerized with white light projected by a rectangular mask from the DMD, then degraded with UV light using a second mask of different geometry. The elastic moduli of the gels were determined with a tribometer after irradiation with white light alone and with white light and UV light. Different UV light irradiation times were used to vary the elastic modulus of the UV-degraded region.

To examine cellular response to the gels, a photomask with a void was used to polymerize PEG via visible light. Rat dorsal root ganglia (DRG) were inserted in the void, and the peptide gel Puramatrix was added to the void. The DRG were cultured for 1 week, then fixed, stained for the neurite microtubule protein βIII tubulin, and evaluated for growth restriction to the Puramatrix-filled void of the PEG gel.

Results

Orthogonal PEG gels exhibited sharp patterns after both visible light polymerization and UV light degradation. After 20 min of irradiation with white light, the gels had an average elastic modulus of 428 ± 15 kPa (Fig. 1A). The gels also decreased in stiffness upon irradiation with UV light following polymerization with visible light. Gels irradiated with UV light for 8 min had an average elastic modulus of 165 ± 16 kPa. The stiffness decreased further with increased UV light irradiation time, as gels irradiated for 16 min with UV light had an average elastic modulus of 94.0 ± 0.60 kPa. This observed decrease in stiffness is likely due to the decrease in crosslink density upon UV-light irradiation. These stiffness measurements also confirmed that the gels were degraded throughout their depth, rather than just at the plane of focus.

DRG cultured in the Puramatrix-filled voids of the PEG gels exhibited robust growth through the gel, but neurite projection was limited to the void (Fig 1B). Because the entirety of the gel was loaded with the cell adhesion protein laminin, the neurite containment was due strictly to the variance in stiffness of the gel.

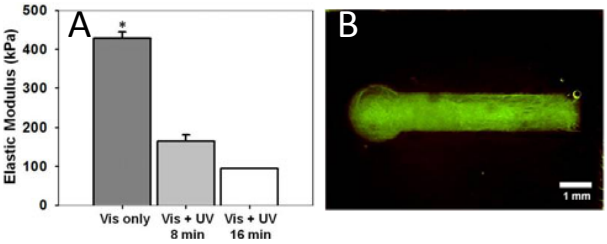

Fig 1. Orthogonal PEG hydrogels. A) Average elastic moduli of gels polymerized with visible light and degraded with UV light, B) DRG neurite growth in void of visible-light polymerized gel filled with Puramatrix.

Conclusions

Using a combination of photoreactive components, we developed a PEG gel of tunable stiffness governed by irradiation times of two different wavelengths of light. The elastic moduli of the visible-light crosslinked region lies just outside the preferred stiffness of central nervous system (CNS) tissue, while the UV-light degraded regions exhibited an elastic modulus similar to that of native CNS tissue. The difference in stiffness was sufficient to contain neurite outgrowth of DRG to the region of lower stiffness, and ongoing experiments will quantify neurite containment in UV-degraded regions of the gels. This orthogonal photoreactive hydrogel has potential as a tunable biomimetic platform for neurite growth models.

References

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2. Bahney CS, et al. Eur Cell Mater. 2011;22:43-55.

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Disclosures

The authors have no financial interests to disclose.