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A tunable synthetic hydrogel system for culture of retinal ganglion cells and amacrine cells

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ABSTRACT

The central nervous system consists of complex groups of individual cells that receive electrical, chemical and physical signals from their local environment. Standard in vitro cell culture methods rely on two-dimensional (2-D) substrates that poorly simulate in vivo neural architecture. Neural cells grown in three-dimensional (3-D) culture systems may provide an opportunity to study more accurate representations of the in vivo environment than 2-D cultures. Furthermore, each specific type of neuron depends on discrete compositions and physical properties of their local environment. Previously, we developed a library of hydrogels composed of poly(ethylene glycol) and poly(L-lysine) which exhibit a wide range of mechanical properties. Here, we identified specific scaffolds from this library that readily support the survival, migration and neurite outgrowth of purified retinal ganglion cells and amacrine cells. These data address important biological questions about the interaction of neurons with the physical and chemical properties of their local environment and provide further insight for engineering neural tissue for cell-replacement therapies following injury.

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1. Introduction

Two-dimensional (2-D) culture systems provide a practical solution for studying survival and neurite outgrowth of neurons in culture; however, three-dimensional (3-D) culture systems can potentially deliver higher degrees of control and organization of cellular environments, more physiologically relevant cell migration, and support the formation of more extensive three-dimensional (3-D) networks of cell–cell interactions [1,2]. Furthermore, cells alone are often not capable of recreating complex tissues on their own although stem and progenitor cells may form 3-D tissues under certain conditions [3]. Nevertheless, on a 3-D scaffold, cells may recapitulate features of complex tissues not otherwise observed in 2-D cultures. Hydrogels are excellent candidates for 3-D scaffolds for ex vivo culture of mammalian cells. Hydrogels demonstrate mechanical properties that parallel the properties of soft tissues [4]. The hydrogel's aqueous environment protects cells, allows for transport of nutrients and metabolite exchange, permits physical, chemical and biological modification, and demonstrates a generally high biocompatibility [5].

In our previous work we developed a library of hydrogels based on poly(ethylene glycol) (PEG) and poly(L-lysine) (PLL) which exhibited a wide range of mechanical properties, a subset of which promoted neural differentiation of neural progenitor cells [6]. Our library of PEG/PLL hydrogels exhibits a wide range of mechanical properties that can be varied independently of the charged PLL component to investigate the role of mechanical modulus on cellular behavior including migration, organization and differentiation [7]. To ask if these scaffolds can support survival and neurite growth of mature neurons, we screened the library of hydrogels, beginning with those identified as good substrates for promoting NSC differentiation. In the appropriate hydrogel environment, we found that acutely purified retinal ganglion cells (RGCs) and amacrine cells (ACs) readily survive and extend extensive neurite outgrowth following seeding onto PEG/PLL-based hydrogel scaffolds.

2. Materials and methods

2.1. Materials

Four-arm PEG (Mn 2000 and 10,000 g mol^{-1}) was obtained from Nektar Therapeutics (Huntsville, AL). PLL (MW 70–150 and 150–300 kDa) and all other reagents were obtained from Sigma

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(St. Louis, MO) and used as received. SpectraPor dialysis membranes (MWCO 1000 and 6000–8000 Da) were obtained from Spectrum Laboratories (Rancho Dominguez, CA) and rinsed thoroughly with deionized water before use.

2.2. Synthesis of activated PEG

Carbamate cross-links between PEG and PLL macromers (Fig. 1A) were created through *N*,*N*-carbonyldiimidazole (CDI) activation of PEG as described previously [6,8]. Briefly, PEG was dissolved in an excess of dioxane at 37 °C. For every hydroxyl present on PEG, a 1:8 M excess of CDI was added. The resulting mixture was stirred under argon for 2 h at 37 °C. Unreacted CDI was removed by dialysis in deionized water for 48 h. The resulting solution was flash frozen in liquid nitrogen and lyophilized for 3 days. Activated PEG was stored in a desiccator at −20 °C.

2.3. Fabrication of hydrogels

All hydrogels were fabricated as 10% w/v polymer in 1× phosphate buffer solution (PBS). A range of PLL and activated PEG ratios were dissolved in 1× PBS, mixed vigorously, and allowed to cure/cross-link at 25 °C for 24–36 h. The PLL and activated PEG ratios chosen were based on a subset of gels from the library of gels previously created [6]. These particular gels were chosen for two reasons: (i) their ability to polymerize without additional chemical modification, and (ii) their ability to influence neural stem cell survival and differentiation [6].

2.4. Hydrogel morphology

To ensure the swollen architecture of the hydrogel would be maintained for imaging, a multistep dehydration process was used. Gels were swollen in 1× PBS for 12–18 h, frozen overnight at −20 °C, placed in a liquid nitrogen bath for 1 h and then lyophilized for 24 h. Gel cross-section samples were mounted and sputter coated with gold for 30 s at 40 mA. The morphology of the hydrogels were then evaluated by scanning electron microscopy (SEM; Philips XL-30 environmental microscope operating at 10 kV).

2.5. Rheology

Elastic moduli for swollen hydrogels were determined as previously described [6]. Briefly, hydrogels were cured and then swollen

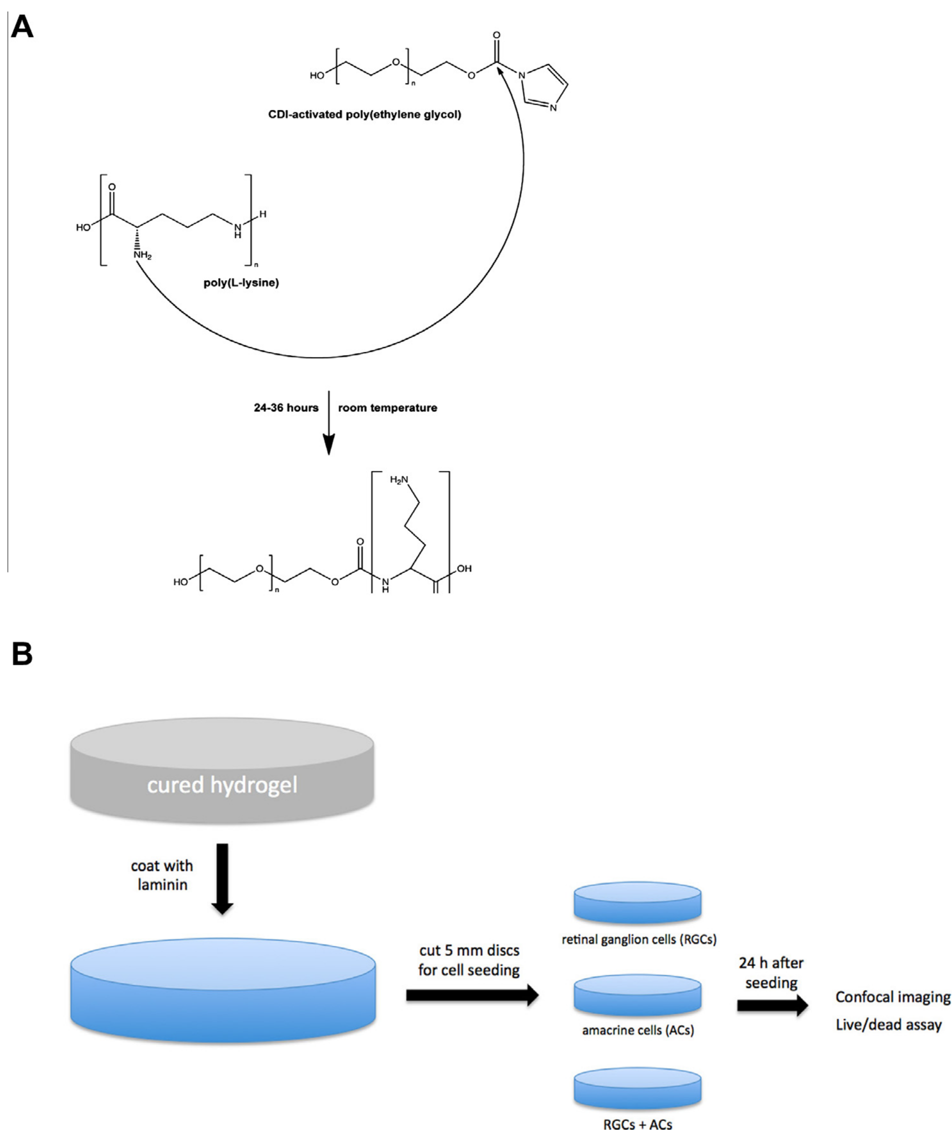

Fig. 1. General experimental procedures. (A) Simplified chemical reaction scheme for the chemically cross-linked hydrogels used in the in vitro studies. (B) Schematic showing the hydrogel preparation procedures and experimental plan for cell seeding and analysis.

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