Dual-gel construct with dynamic control of soluble chemotactic cues for the study of neural cells
O. Kuzura1,2, C. Petersen1,3, V. Rengarajan1, and R. Perez-Castillejos1
1New Jersey Institute of Technology, Newark, NJ, 2Grinnell College, Grinnell, IA, 3Case Western University, Cleveland, OH

Introduction: Complex neural networks form with great precision because environmental stimuli—including gradients of soluble, molecular chemotactic cues—guide the growth of neural processes [1]. Dual hydrogel constructs have been developed to study the outgrowth and guidance of neurites within in-vitro environments that resemble the neural tissue. The widespread use of these dual-gel constructs, however, is limited because they require complex fabrication methods and expensive equipment [2]. Our goal here was to develop a simple, inexpensive fabrication process for producing dual-hydrogel constructs to study neurite outgrowth under dynamically varying gradients of diffusing cues.

Materials and Methods: Our device consists of a a poly(ethylene glycol) diacrylate (PEGDA) hydrogel frame, which holds 0.15% PuraMatrix hydrogel in its interior (Fig. 1b). The frame results of crosslinking PEGDA under UV light through a mask produced by shaping black photographic film with a programmable cutter (Silhouette Cameo®), Fig. 1a. A methacrylated coverslip supported the PEGDA frame preventing it from slipping during use. The design of the PEGDA device features two perpendicular channels connecting to (i) two circular chambers to deliver soluble cues and (ii) two large triangular reservoirs for cell culture medium. Cells will be cultured in the chamber where the two channels cross, in the center of the device. To control the soluble environment of cells in the channels, we created cue-containing PEGDA tablets that fit tightly inside the circular chambers—see pink tablet (red arrow) on the left of Fig. 1b. We then rinsed the space inside of the PEGDA frame and filled it with PuraMatrix solution [3], which gelled upon addition of growth medium.

Characterization of molecule diffusion in the PuraMatrix-PEGDA construct. We first soaked the PEGDA tablets for 1 h in PBS (phosphate buffered saline, control) or in 0.1 mM solutions of fluorescently labeled dextran of various molecular weights (3, 40, and 70 kDa) representative of the various sizes of molecular cues found in vivo. We then placed the tablets into the chosen circular chambers and imaged the whole construct by fluorescence microscopy (Fig. 1c). Fluorescence profiles were determined from the images using ImageJ (Fig. 1d).

Results and Discussion: We successfully produced a dual-hydrogel construct using a fast protocol and inexpensive equipment that is accessible to every biomedical lab. The combination of two hydrogels provides advantages for the study of neural cells. On one hand, PEGDA is conducive to neurite growth and contains neurites within the channels. On the other hand, 0.15% PuraMatrix presents a mesh size that supports neurite outgrowth, and results in the neural processes extending along the channels of the device. We were able to culture DRGs in our dual-gel devices for up to 7 days, as indicated by Live/Dead assays. Diffusion of molecules (fluorescently labelled dextran) from the tablets in the circular chambers allowed for successful control of the levels of soluble cues in the channels filled with PuraMatrix (Fig. 1d). Replacement of a dextran-loaded tablet by a PBS tablet reduced fluorescence levels to basal levels.

Conclusions: Our device is a versatile platform for conducting neurite outgrowth studies under gradients of a wide array of guidance proteins, as represented by the studies with fluorescently labeled dextran presented above. It is possible to create multiple gradients simultaneously—as it occurs in vivo—by placing tablets with different contents in the chambers. Replaceable tablets enable control over steepness and duration of a protein concentration gradient. Additionally, PuraMatrix can be modified with extracellular matrix components that make the construct more closely model in-vivo conditions. Because the protocols of fabrication and use of our dual-gel devices are simple and inexpensive, we expect our work to be easily replicated by researchers in multiple biomedical labs, even those without a background in microfabrication and photolithography.

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