Photoreactive Interpenetrating Networks as Tunable Scaffolds for Neurite Growth

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Introduction

Tunable matrices that support axon growth are essential for development of neural tissue models, drug discovery, and regenerative therapies. We previously described a 3D dual hydrogel system to model neurite growth, consisting of cell growth permissive (Puramatrix (PM)), and restrictive (Polyethylene glycol diacrylate (PEG)) regions [1]. An interpenetrating polymer network (IPN), two polymer networks physically entangled but not chemically crosslinked, provides the advantage of using characteristics from two polymers in one network. This work describes novel hydrogels that utilize photocrosslinkable methacrylated hyaluronic acid (Me-HA) or dextran (Me-Dex) and PM systems that promote cellular growth and allow the adjustment of mechanical and structural properties by varying the degree of methacrylation or binding of proteins.

Methods

Me-HA and Me-Dex with different degrees of methacrylation (Me-HA: 32% and 90%, Me-Dex: 20%) were synthesized [2]. photocrosslinkable solutions of Me-HA (4% w/v) and Me-Dex (10% w/v) were prepared containing 0.4% (w/v) Irgacure 2959 and 0.01% (v/v) N-vinyl-pyrrolidinone in H_2O, and the PM solution was prepared by adding 1µg/ml laminin to 1% (v/v) PM solution in H_2O. To fabricate the IPN system, Me-HA or Me-Dex and PM solutions were mixed 1:1 and 2:1 accordingly. Each IPN system was characterized for bond formation, scanning electron microscopy (SEM), viscoelastic and swelling behavior and gel distribution.

A photocrosslinkable solution of 10% (w/v) PEG in PBS was crosslinked in specific geometries using UV light projected by a digital micromirror device (DMD). For the Me-HA IPN system, in order to study the influence of stiffness on neurite growth, IPN_{90} and IPN_{32} solutions were added stepwise to opposite sides of a bipolar PEG micromold. Me-HA IPN was polymerized using UV light (14 min), and soaked for 1 hr in PBS for PM assembly. For the Me-Dex IPN system, a rectangular PEG mold was filled with the photocrosslinkable solution and, using the DMD and desired photomask, a channel was patterned into the gel with a constant irradiation time of 90 sec. Gels then soaked in PBS for 1hr. The non-irradiated region forms a Semi-IPN (S-IPN) with only PM developing a hydrogel network.

Dorsal root ganglia (DRGs) from E15 rat pups were inserted into the gels. The DRGs were cultured for 7 days before fixing and stained for βIII tubulin, glial cell markers and DAPI. Fluorescent microscopy and confocal imaging was done to study volume and depth of cell growth and evaluate neurite extension (Fig. 1).

Results

Hydrogel network formation and entanglement were examined using FT-IR, SEM (Fig. 1), and gel distribution studies. These studies confirmed the uniform formation of each network, and that the two networks homogeneously entangled within the IPN systems. SEM showed that the IPN forms fibrillar porous structures which are more entangled after addition of PM and the microstructure was reinforced by incorporating PM in Me-HA network (Fig. 1). Mechanical properties of the hydrogel systems were evaluated using rheology (Fig. 1), tribometery and swelling behavior. Higher crosslinking density of methacrylated networks in the IPNs augmented the elastic and compressive moduli, as it reinforced the network on the molecular scale. Fewer methacrylate groups in IPN systems led to a higher swelling ratio and less stiff environment that encouraged axonal growth and extension. In contrast, S-IPN systems were only held together by the PM network, resulting in looser architecture and more favorable neurite growth environment than IPNs with multiple polymer networks, but lacked the ability to become crosslinked into the surrounding mold.

We fabricated a high throughput 3D hydrogel model for neuronal studies with tunable cues. The two models presented here show a strong correlation between increasing neurite outgrowth and decreasing stiffness. Using photolithography, we created a hydrogel with both IPN (exposed to UV light) and S-IPN (not exposed to UV light) regions for directed neurite growth. Future studies will examine the effect of bound proteins on neurite growth in the IPN systems.

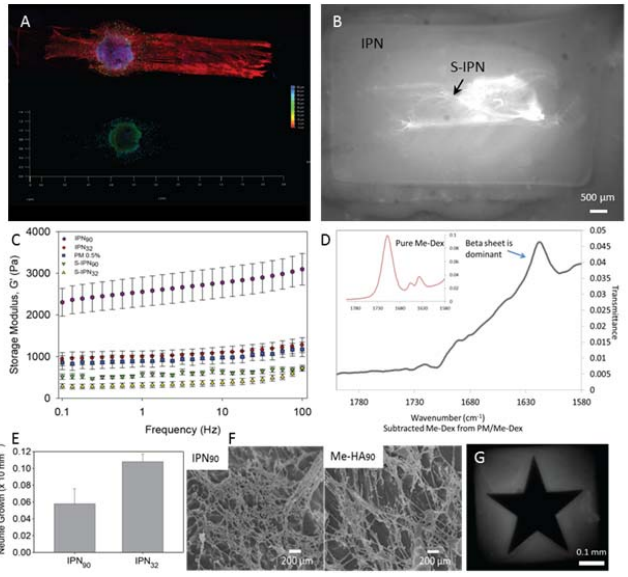

Figure 1. A) DRG neurite growth in dual hydrogel constructs: β III tubulin-positive neurites (red), DAPI-stained nuclei (blue) and glial cell markers (green). B) Neurite growth contained in micropatterned S-IPN region of Me-Dex IPN system. C) Storage moduli (G') of gels. D) FT-IR: β-sheet formation organizes into network both in PM with or without Me-Dex after soaking in PBS. E) Volume of growth in different Me-HA/PM IPNs. F) micropatterned Me-HA IPN. G) SEM: A uniform and more dense Me-HA IPN structure.

Conclusions

We designed an *in vitro* system with controllable mechanical and structural properties. The stiffness can be tuned easily by altering the degree of methacrylation of HA or irradiation time. This integrated hydrogel model is an effective system for promoting neurite outgrowth in a spatially defined manner within a 3D environment.

References

1-Curley JL, et al. J Biomed Mater Res. 2011;99:532-43.
2-Bencherif SA, et al. Biomaterials. 2008;29:1739-1749.

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