Photoreactive Interpenetrating Network with Tunable Stiffness as a Scaffold for Neurite Growth

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Introduction

Tunable matrices that support axon growth are essential for development of neural tissue models for drug discovery, for research in neural growth and regeneration, and for development of regenerative therapies. We previously described dual hydrogel micropatterning as a basis for neural growth models [1]. Micropatterned polyethylene glycol diacrylate (PEG) was used as a micromold, while the self-assembling peptide gel Puramatrix (PM) was used as a cell growth matrix. While PM promotes robust neurite outgrowth, it does not facilitate formation of gels with variable stiffness, which is a parameter known to influence neurite growth. Furthermore, there is no integration of PM with the PEG gel, often leading to separation of the dual hydrogels.

We describe an integrated 3D neurite growth model using photocrosslinkable, methacrylated hyaluronic acid (Me-HA) and PM to form an interpenetrating network (IPN) inside the PEG micromold. Utilizing an IPN was shown to rectify the problem of gel separation, resulting in a more integrated system. Furthermore, we can proceed to change either the degree of methacrylation of the Me-HA or the UV-light irradiation time to alter stiffness, providing two independent parameters for designing neurite outgrowth models.

Methods

Me-HA with two different degrees of methacrylation were synthesized according to a published protocol [2]. Compressive moduli of the synthesized Me-HA polymers with either 32% or 90% methacrylation were measured *in situ* at different irradiation times using a tribometer with a 1.25 mm diameter probe.

A photocrosslinkable solution of 10% (w/v) PEG (MW = 1000 Da), and 0.5% (w/v) Irgacure 2959 (I-2959) in PBS was crosslinked in specific geometries with UV light projected by a digital micromirror device [1].

For the IPN system, 1% (w/v) Me-HA solutions contained 0.4% (w/v) Irgacure 2959 and 0.01% (v/v) N-vinyl-pyrroldinone in H_2O [3] 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 and PM solutions were mixed 1:1 and added to the voids in the PEG hydrogels. A constant irradiation time of 14 mins with UV light was used to polymerize the Me-HA, after which they were left for an hour in PBS for PM to form a network. To evaluate the stability of the dual hydrogel system and the integration of IPN and PEG the constructs were soaked in 4ml PBS in a 150 rpm shaker set at 37°C overnight.

Neural growth studies were carried out with dorsal root ganglia (DRGs) dissected from E15 embryonic rats inserted into the non-irradiated region of the gel. The DRGs were cultured for 7 days before fixing and staining for the neurite microtubule protein βIII tubulin, and the area of cell growth was evaluated with image analysis using Image J.

Results

This research describes a dual hydrogel system which allows variation of material stiffness due to a Me-HA/PM IPN, allowing *in vitro* modeling of environments that may be found in neurite growth scenarios of varying stiffness. This system allows us to introduce a range of stiffness simply by changing either the irradiation time or methacrylation degree in Me-HA and study the effect of this change on the neurite growth. Characterization of the Me-HA/PM IPN hydrogels showed that the elastic modulus of the gels increased with higher substitution degree in Me-HA. DRGs which were cultured in the IPN region grew successfully with minimal outgrowth to the PEG scaffold. The two models that have been tested show a strong correlation between increasing cell growth and increasing stiffness, as can be seen in Figure 1. In the specific range of stiffness that neurons tend to grow, significant increase in the stiffness leads to a significant increase in neuronal growth.

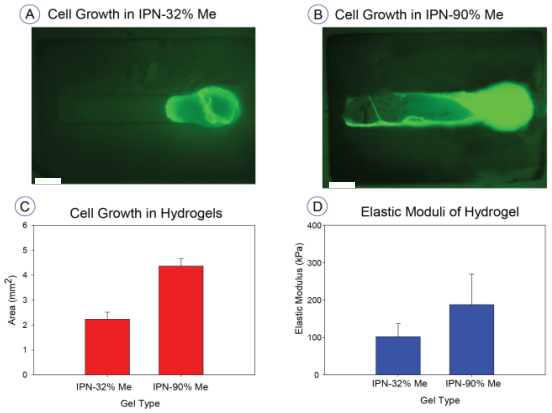

Fig 1. Micropatterned IPN/PEG for 3D neurite outgrowth with A) less growth in less stiff IPN segment with DRG explant stained with β-III Tubulin (green), and B) robust growth in the stiffer system. C) Graph of area covered by cells in each hydrogel and D) graph of elastic moduli of IPN with 32% and 90% of methacrylation correlate increasing stiffness with increasing cell growth. Scale bar = 500 µm.

This study also showed that the IPN was integrated into the PEG scaffold and did not detach after incubation with shaking, which had previously been observed with PM alone.

Conclusions

The integration of an IPN and PEG scaffold provides *in vitro* systems with controllable stiffness for studying neurite growth. The stiffness can be tuned easily by altering the ratio of Me-HA/PM and the degree of methacrylation of HA. This integrated hydrogel model is an effective system for promoting neurite outgrowth in a spatially defined manner within a 3D environment.

References

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Disclosures

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