A Dual Hydrogel System to Prevent Axonal Overgrowth in 3D Neural Tissue Models

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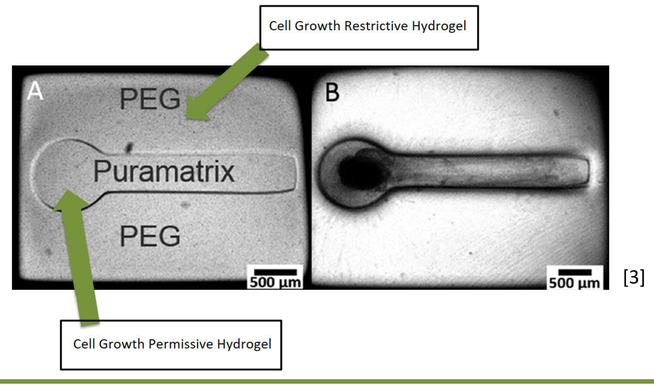
Background

Big Problem: 10+ years and $2.6B to bring a drug to market [1] and 2D models do not accurately recapitulate in vivo morphology or functionality [2]

Proposed Solution: Creation of a 3D microphysiological model that accurately mimics in vivo physiology to provide preclinical indications of clinical drug success

Technique: Combination of localized organization seen in cell spheroids with distant projections that are guided by a dual hydrogel structure

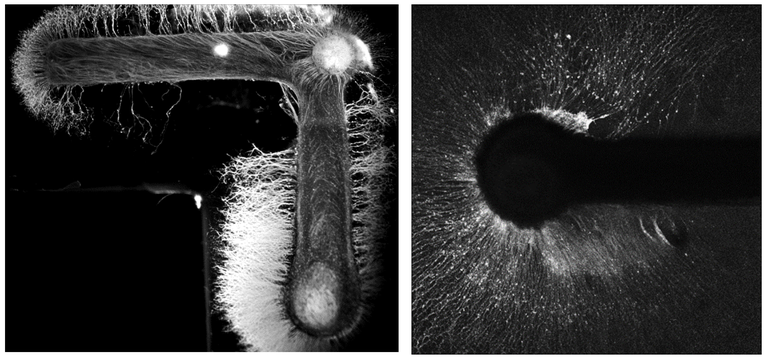
Dual hydrogel structure is constructed from an outer cell growth restrictive gel (PEGDA) and an inner cell growth permissive gel (Matrigel, GelMa, Puramatrix)



[3]

Problem

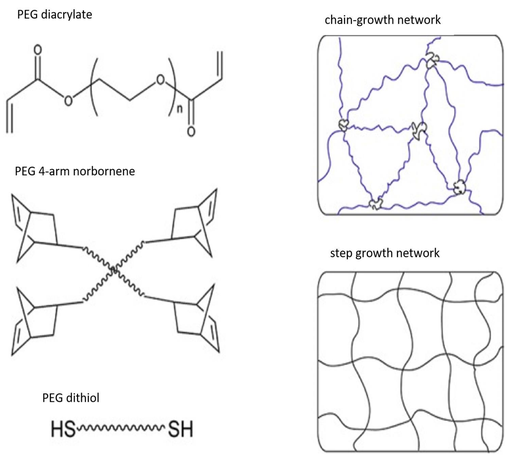
- Axon overgrowth occurs after several weeks
- Growth occurs on top of the restrictive PEG gel



'B3 Tubulin Staining

Proposed Solution

- Switch the outer gel from PEGDA to 4-arm PEG norbornene and a PEG Dithiol crosslinker
- The benefits of switching to 4 arm PEG norbornene include known molecular weight between crosslinks, a more controlled and organized step growth network instead of a disorganized chain growth network, known swelling rates, use of less photoinitiator and the ability to take advantage of the thiol-ene click chemistry to add molecules with a thiol group
- Incorporation of axon growth inhibiting protein, Semaphorin 3A after thiolation



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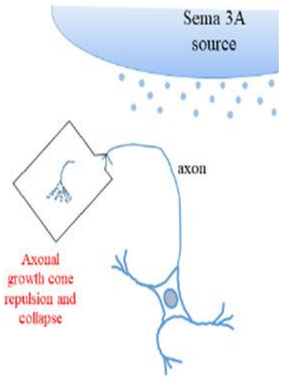

Axon repulsion due to Semaphorin 3A
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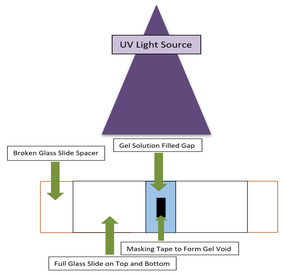
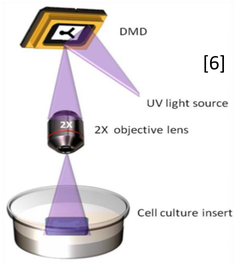
Methods

- Thiol-ene click chemistry
- UV exposure crosslinking the PEG solution





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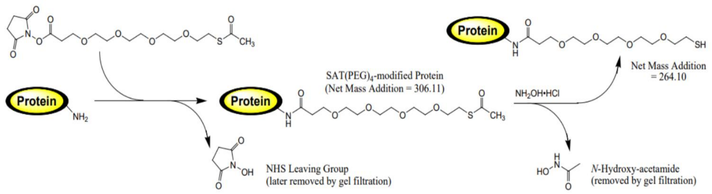
Methods

- Thiolate protein using SAT(PEG)4
- Incorporate thiolated protein into PEG crosslinking network



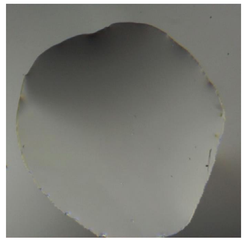
Results

- Formed a workable gel with the 4 arm PEG norbornene and the PEG Dithiol crosslinker

Best Formulation:

- 4:1 Ratio of PEG dithiol to PEG norbornene
- 20% PEG solution (w/v)
- 1.1 mM LAP photoinitiator
- 5 min of exposure to UV light at 385 nm

- Thiolated fluorescent BSA



Future Work

- Testing with varying protein concentrations
- Imaging of the thiolated fluorescent BSA included in the PEG crosslink network before and after washing
- Imaging of unthiolated fluorescent BSA in the PEG gel before and after washing
- Possible new protein detection method
- Inclusion of dorsal root ganglia cell spheroids
- Inclusion of the Semaphorin 3A (SEMA 3A) protein to inhibit axonal outgrowth

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