The development of injectable photopolymerizable Gelatin methacryoloyl hydrogel with tunable properties for axonal regeneration following spinal cord injury

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Background and motivation

- More than 86,000 people in Canada live with a spinal cord injury (SCI).
- Individuals with SCI suffer from the partial or complete loss of mobility, physiological and sensory functions.
- The lack of regeneration at the injury site is caused by inflammation, formation of a cavity and a glial scar.
- Neuroregenerative therapies hold promise in restoring the structural and functional integrity of the spinal cord.
- They include biomaterials which could be injected at the injury site and provide a supportive substrate for axonal growth.
- Hydrogel biomaterials can be fabricated using photopolymerization reaction that offers fast hydrogel formation, and an accurate temporal and spatial control over the reaction.

Aim

To develop a hydrogel-based biomaterial that can be injected at the site of SCI to create a permissive environment for axonal regeneration.

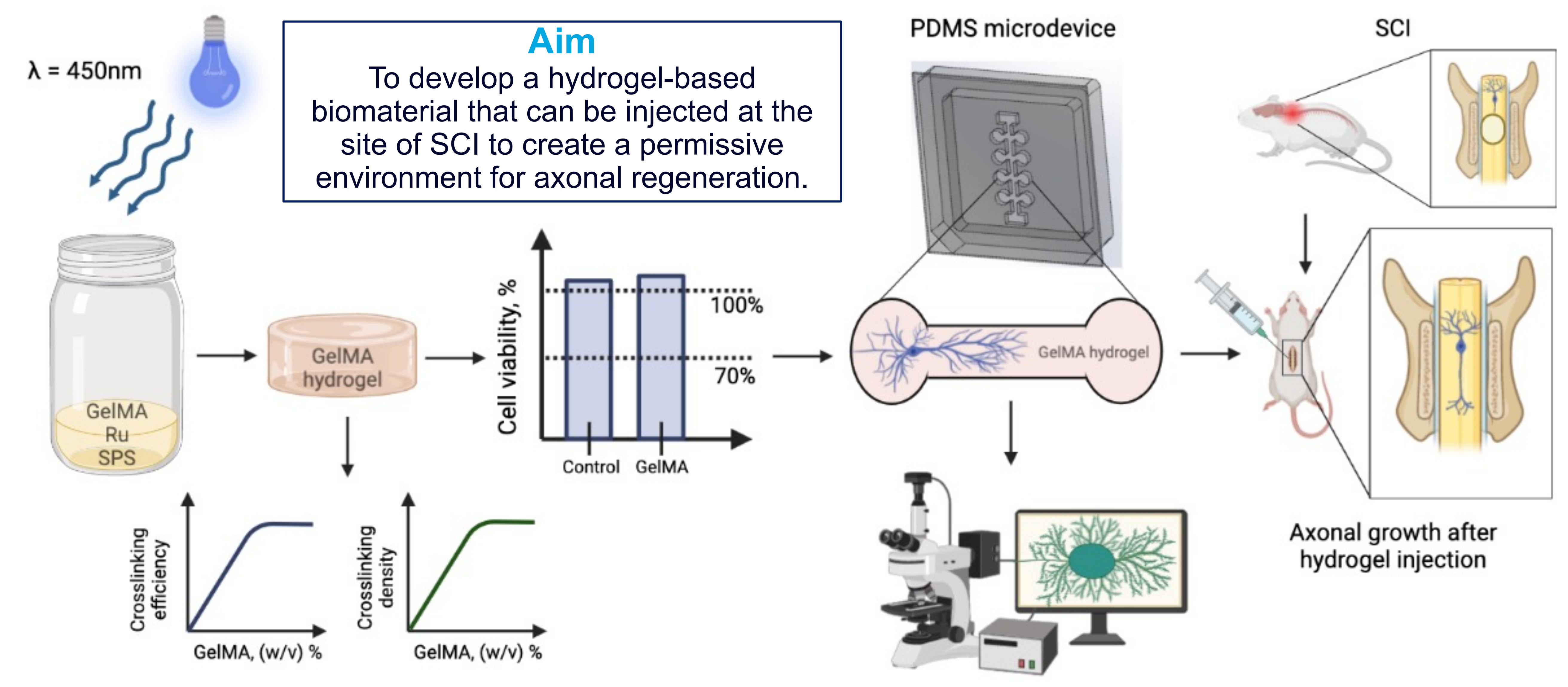
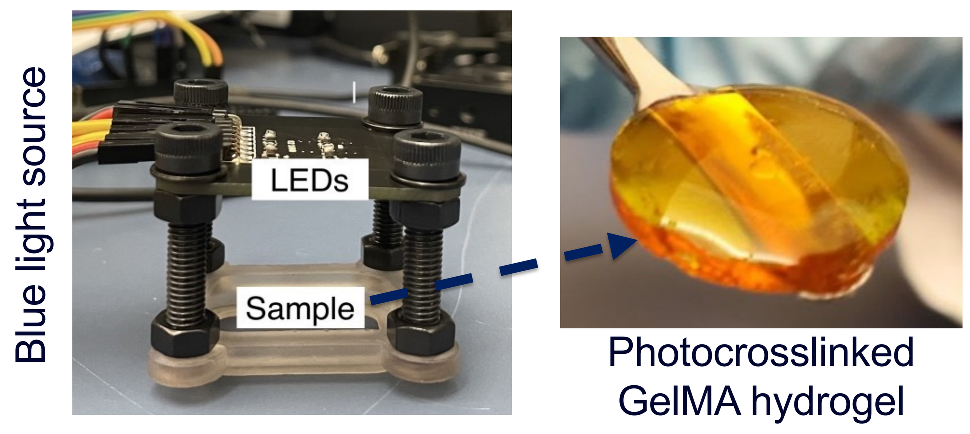


Illustration created using Biorender.com

Methodology

Objective 1: Design and characterization of photopolymerizable Gelatin methacryoloyl (GelMA) hydrogel.

- Photoinitiator ruthenium (Ru) and sodium persulfate (SPS) in 1:10 ratio was used to polymerize 6% (w/v in PBS) GelMA hydrogels through the exposure to blue light (λ = 450 nm, 60s).



- Crosslinking efficiency of hydrogels was evaluated by calculating sol fraction (SF) parameter using the *Equation 1*.

Photo-polymerization ($m_{initial}$) → Freeze-drying ($m_{initial, dry}$) → 37°C, 24h in PBS → Freeze-drying (m_{dry})

$$((m_{initial, dried} - m_{dry})/ m_{dry}) \times 100\% \quad \textit{Eq. 1}$$

- Crosslinking density of hydrogels was evaluated by calculating the equilibrium swelling ratio (SR) using *Equation 2*.

Photo-polymerization ($m_{initial}$) → Swelling ($m_{swollen}$) → Freeze-drying (m_{dry})

$$(m_{swollen} - m_{dry})/ m_{dry} \quad \textit{Eq. 2}$$

Internal parameters	External parameters
Degree of functionalization (DoF) 50% vs 80%	Light exposure power 10 mW vs 50 mW
GelMA concentration	Light exposure time
Ru/SPS concentration	

***Table 1**. GelMA composition (internal) and photocrosslinking conditions (external) parameters that affect hydrogel biocompatibility and material properties, parameters explored in this work are highlighted in red.*

Objective 2: Evaluate biocompatibility of GelMA hydrogels and axonal growth in 3D cell culture.

- Dorsal root ganglion (DRG) explants were isolated from laboratory rat spinal cord and encapsulated in the hydrogel of choice in a SCI-on-a-chip PDMS microdevice.
- DRGs were cultured for 14 days, then were fixed, stained with neuron-specific Tuj-1 antibody, and imaged using confocal fluorescent microscope.
- Ultimatrix (8 mg/ml) hydrogel was used as a positive control. GelMA 6% Ru/SPS 0.2/2 mM hydrogel was applied in the experimental group.
- GelMA crosslinking conditions (exposure to blue light with and without Ru/SPS) was applied to the Ultimatrix hydrogel to evaluate the biocompatibility.

Results

- Application of higher power levels of blue light for crosslinking (50mW vs 10 mW) had significantly increased crosslinking efficiency ($p=0.0258$) in GelMA 80%DoF hydrogels.
- Higher DoF in GelMA leads to a decrease in crosslinking density of hydrogels, as demonstrated by statistically significant increase in SR in the case of 80%DoF-50mW GelMA hydrogels compared to the 50%DoF-50mW ($p=0.0048$).
- SR results indicated a statistically significant increase in crosslinking density for both 50%DoF and 80%DoF hydrogels crosslinked using 10mW compared to 50mW ($p=0.0449$ and $p<0.001$ respectively).

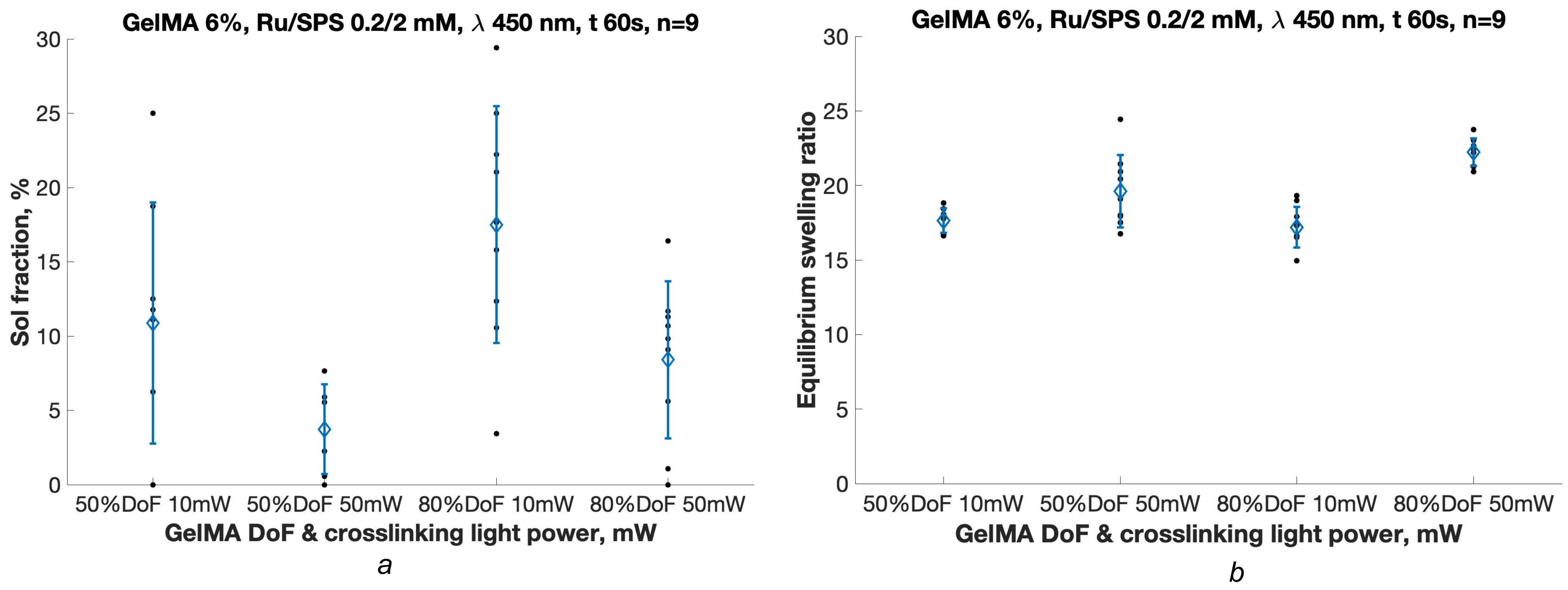
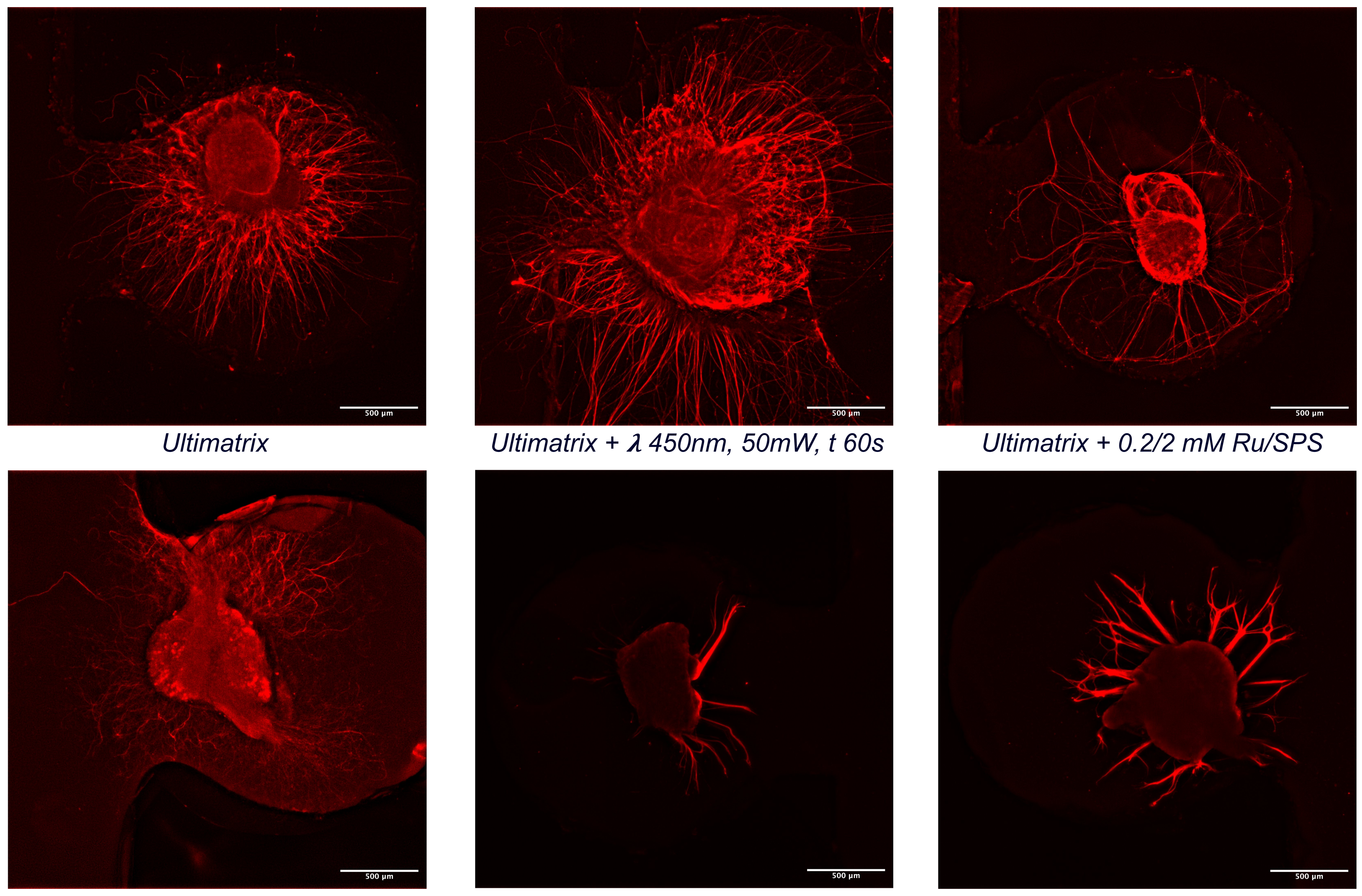


***Figure 1**. Sol fraction (**a**), and swelling ratio (**b**), results for hydrogels prepared using 50%DoF or 80%DoF GelMA formulation and two different levels of light power (10mW or 50mW) for photocrosslinking.*



Ultimatrix | *Ultimatrix + λ 450nm, 50mW, t 60s* | *Ultimatrix + 0.2/2 mM Ru/SPS*

Ultimatrix + 0.2/2 mM Ru/SPS, λ 450nm, 50mW, t 60s | *GelMA 6% **50%DoF** 0.2/2 mM Ru/SPS, λ 450nm, 50mW, t 60s* | *GelMA 6% **80%DoF** 0.2/2 mM Ru/SPS, λ 450nm, 50mW, t 60s*

***Figure 2**. Fluorescent images of Tuj 1-labelled DRG neurite extensions fixed on day 14 of culture in various hydrogels.*

- The addition of Ru/SPS to Ultimatrix led to a decrease in the number of neurites compared to the Ultimatrix control.
- 6% GelMA hydrogels have supported the growth of neurites from DRGs.

Future work will focus on further investigation into the possible cytotoxic effects from photopolymerization reaction, as well as mitigation methods; and hydrogel stiffness tuning.

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SBME UBC SCHOOL OF BIOMEDICAL ENGINEERING

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