PHOTOCROSSLINKABLE GELMA HYDROGELS SUPPORT NEURITE OUTGROWTH IN A MICROSCALE IN VITRO SPINAL CORD INJURY MODEL

Iryna Liubchak[1,2], Nasrin Zohreh[1,2], Alex Pieters[1,2], Paul Juralowicz[1,2], Tanya Bennet[1,2], Tara M. Caffrey[1,2], Karen C. Cheung[1,2,3]

[1]School of Biomedical Engineering, [2]Centre for Blood Research, [3]Department of Electrical and Computer Engineering, University of British Columbia

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.
- 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.
- Photocurable biomaterials offer fast and efficient light stimulus-controlled hydrogel formation at the injury site.

Aim

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

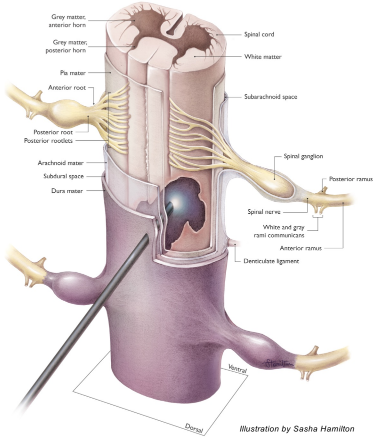
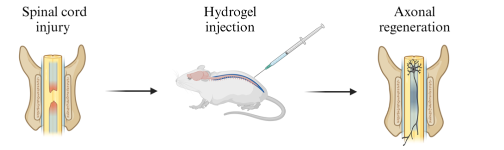
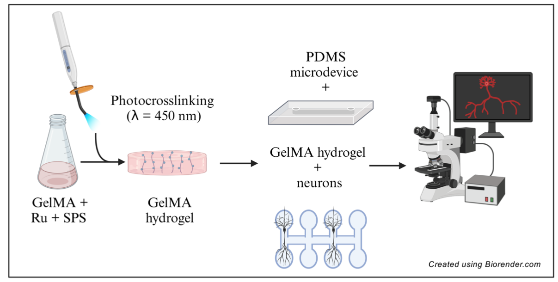


Illustration by Sasha Hamilton





Methodology

Objective 1: Design and characterization of visible light-curable Gelatin methacrylate (GelMA) hydrogel.

- Photoinitiator sytem composed of ruthenium (Ru) and sodium persulfate (SPS) in 1:10 ratio.
- 3% and 6% GelMA with either 50% or 80% degree of methacrylic group functionalization (DoF) was used.
- Hydrogel formulation was prepared in PBS or cell culture media as a solvent.
- Hydrogels were fabricated through the exposure to blue light (λ = 450 nm) for 60s.

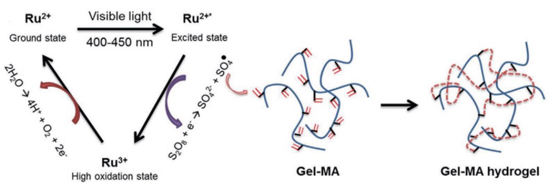


Image adapted from Lim, K. S. et al. Macromol. Biosci. 2019, 19, 1900098

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

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

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

- 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})

$$SR = (m_{swollen} - m_{dry}) / m_{dry} \quad \text{Eq. 2}$$

***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 rat spinal cord and encapsulated in the hydrogel of choice in a SCI-on-a-chip PDMS microdevice.
- DRGs were cultured for 7 days, then fixed, stained with neuron-specific anti-beta-III-tubulin antibody, and imaged using fluorescent microscope.

Ongoing and future work

- further hydrogel composition tuning through the adjustments in GelMA concentrations and crosslinking conditions;
- in vivo injection of GelMA hydrogels into the acute and chronic spinal cord injury animal models.

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Results

Hydrogel characterization

- Application of higher blue light power levels for crosslinking (50 mW vs 10 mW) had significantly decreased SF indicating higher crosslinking efficiency in GelMA 80%DoF hydrogels.
- Higher DoF in GelMA leads to a decrease in crosslinking density of hydrogels, shown by statistically significant increase in SR in the case of 80%DoF-50 mW GelMA hydrogels compared to the 50%DoF-50 mW.
- This was confirmed via the mechanical rheological testing of the hydrogels: storage modulus (G') of the 80%DoF GelMA hydrogel was lower compared to the 50% DoF (595 ± 21.4 Pa vs 1132 ± 95.2 Pa respectively (n=3), both 6% w/v and crosslinked using 50mW power).
- SR results indicated a statistically significant increase in crosslinking density for both 50%DoF and 80%DoF hydrogels crosslinked using 10 mW compared to 50 mW.

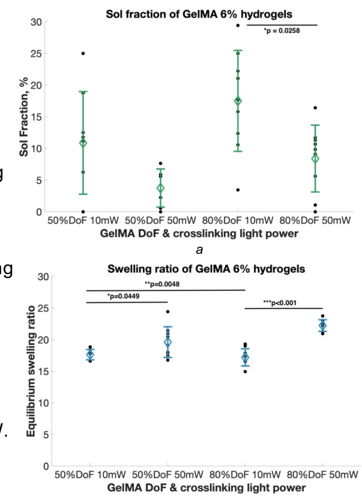

***Figure 1**. Sol fraction (**a**), and swelling ratio (**b**), results for hydrogels (n=9) prepared using 50% or 80% DoF GelMA formulation and two different levels of light power (10 mW or 50 mW) for photocrosslinking. Statistics used were one-way ANOVA and multiple comparison tests.*

Neurite growth in GelMA hydrogels

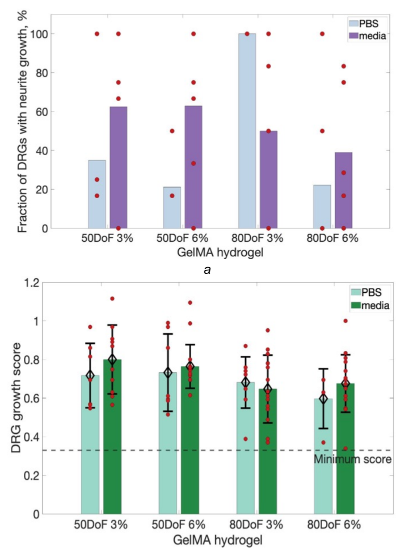

***Figure 2**. Fraction of neurite growth from DRGs in various hydrogels, where data points represent the mean fraction of individual DRG batches (**a**), and the DRG growth score assigned based on the length and spread of neurites and normalized by growth score achieved in the Ultimatrix control in individual DRG batches (**b**).*

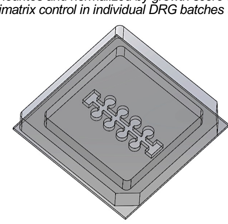

***Figure 3**. PDMS microdevice – in vitro spinal cord injury model used for 3D cultures of DRG explants in various GelMA hydrogels.*

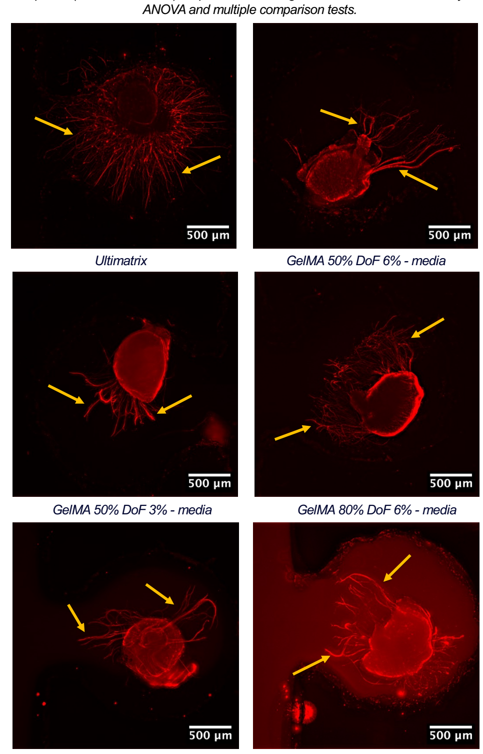

***Figure 4**. Fluorescence images of DRG neurite extensions in various hydrogels.*

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