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

Iryna Liubchak[1,2], Emad Naseri[1,2], Tanya Bennet[1,2], Tara M. Caffrey[1,2], Karen C. Cheung[1,2,3]

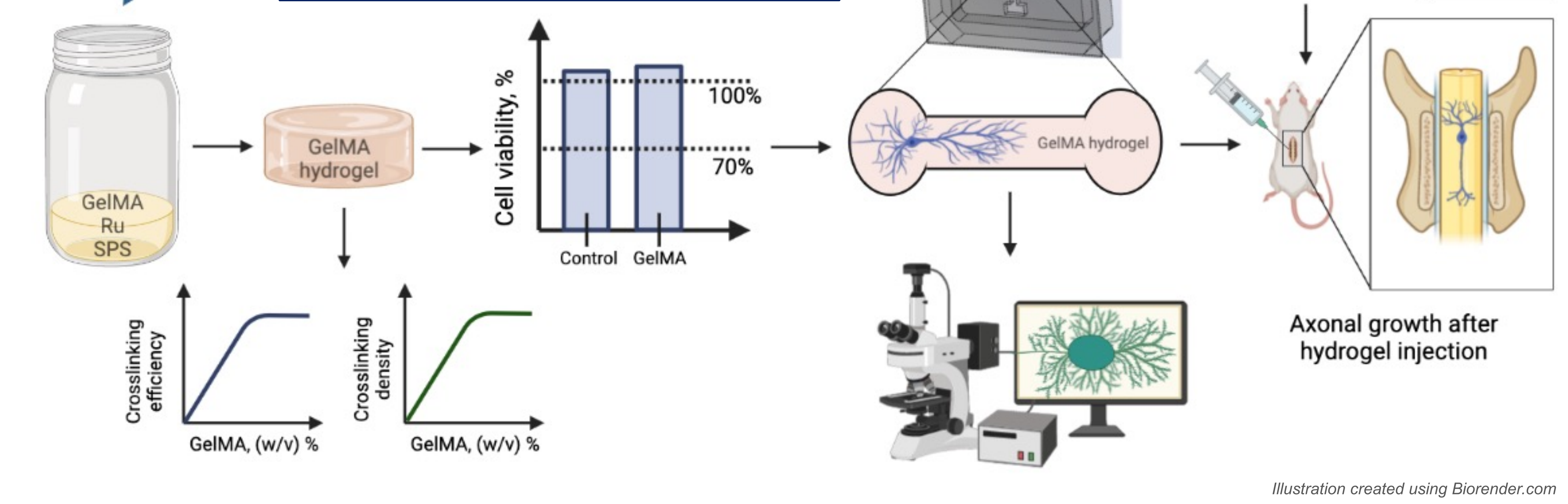
[1]School of Biomedical Engineering, [2]Centre for Blood Research, [3]Department of Electrical and Computer Engineering, University of British Columbia

Background and motivation

- More than 86,000 people in Canada live with a spinal cord injury (SCI)[1].
- 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 easy temporal and spatial control over the reaction.

Aim

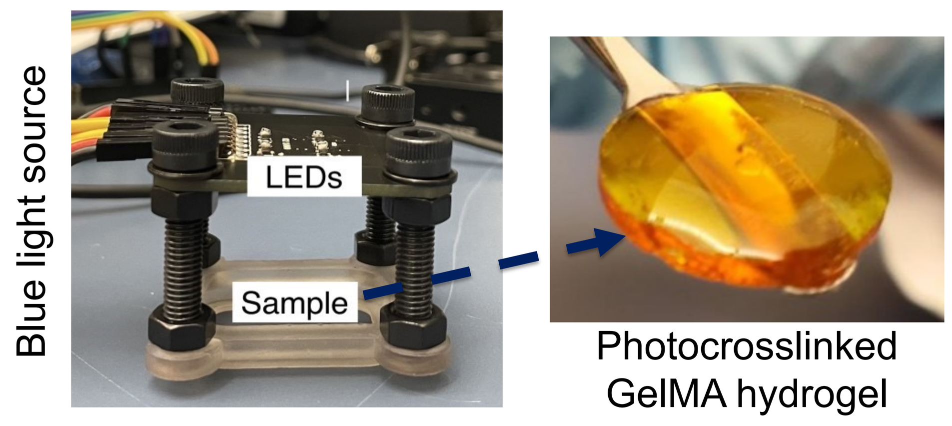
To develop a hydrogel-based biomaterial that can be injected at the site of SCI to create a permissive environment for axonal regeneration.



Methodology

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

- Photoinitiator (ruthenium (Ru) and sodium persulfate (SPS)[2] in 1:10 ratio) was used to polymerize GelMA hydrogels through the exposure to blue light (**λ** = 450 nm, 60s, 50 mW).



- 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})

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

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

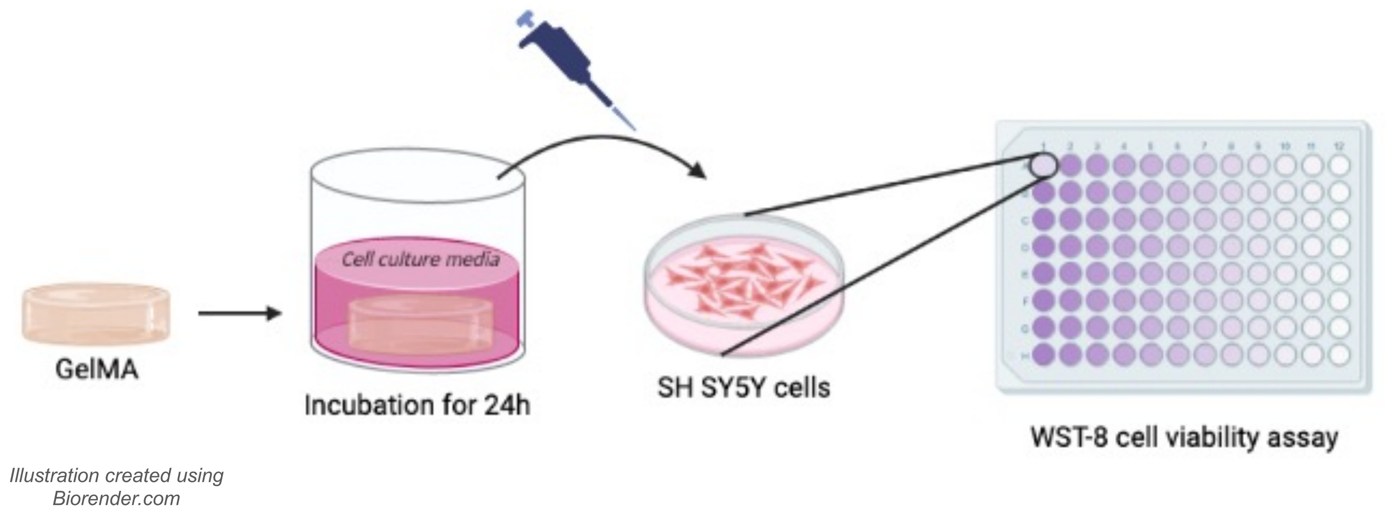
Photo-polymerization ($m_{initial}$) → Swelling ($m_{swollen}$) → Freeze-drying (m_{dry})

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

- An impact of different parameters (GelMA type and concentration, Ru/SPS concentration) on SF and SR values of hydrogels was explored.

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

- Indirect cytotoxicity test was performed on SH SY5Y neuroblastoma cells using hydrogels with high and low sol fraction values according to the ISO protocol[3].



- Dorsal root ganglion (DRG) explants were encapsulated in GelMA 6% 0.2/2 Ru/SPS hydrogels in a SCI-on-a-chip PDMS microdevice, and the growth of neurites in the hydrogel was tracked for 14 days.

Results

Hydrogel characterization

- GelMA hydrogels prepared using type A (porcine) gelatin had significantly higher crosslinking efficiency ($p=0.0023$) compared to type B (bovine) GelMA, as well as higher crosslink density (SR=18.5 and 33.4 respectively, $p<0.001$).
- Increasing GelMA concentration had a significantly higher impact on SF value ($p=0.0047$) compared to the Ru/SPS concentration ($p=0.15$) and improved hydrogel crosslinking efficiency.
- Increasing GelMA and Ru/SPS concentration decreased SR value of hydrogels and improved crosslinking density.

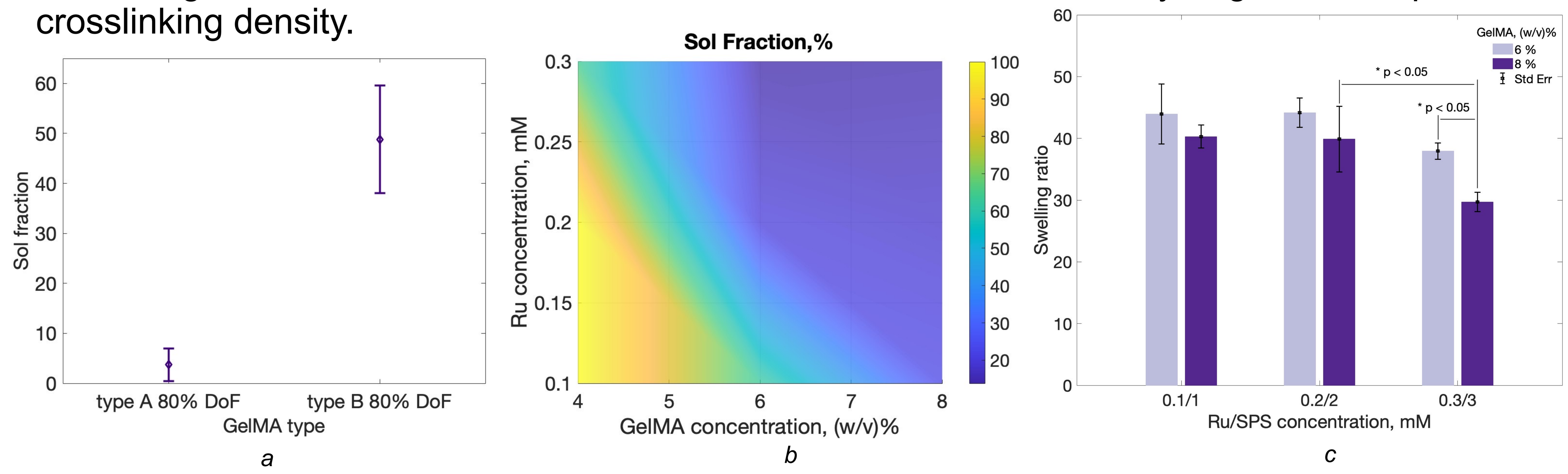
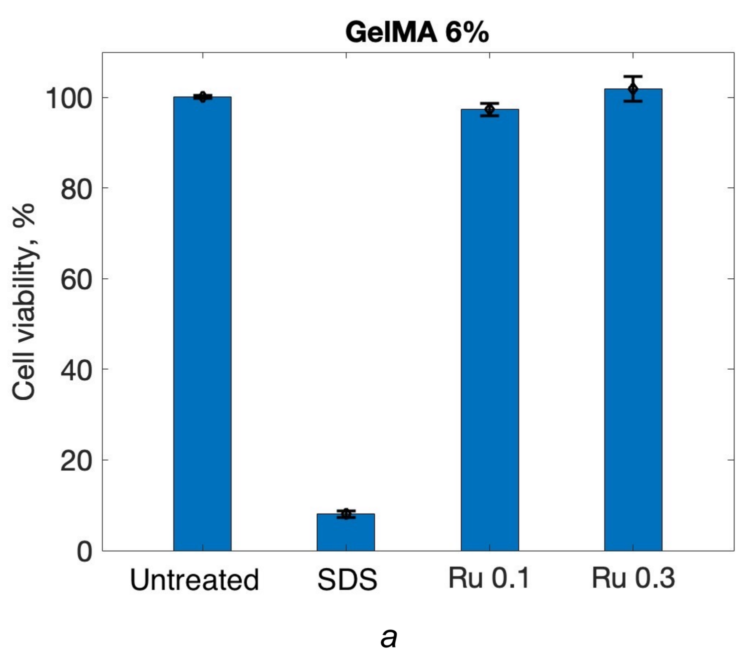


*Figure 1. Type A GelMA with 80% degree of functionalization (DoF) allows to crosslink 6% GelMA hydrogels more efficiently compared to the type B GelMA with 80% DoF (**a**); surface contour plot representing interactions between two concentration parameters (GelMA and Ru/SPS) and their impact on SF value in type B GeMA hydrogels (**b**); higher GelMA and Ru/SPS concentrations lead to a statistically significant decrease in the SR of type B GelMA hydrogels.*

Hydrogel biocompatibility and axonal growth

- Viability levels of SH SY5Y cells treated with media extracts from GelMA hydrogels were above 70% level indicating the absence of cytotoxicity[3].
- DRG demonstrated the growth of neuron extensions in GelMA hydrogels for 2 weeks of 3D cell culture.



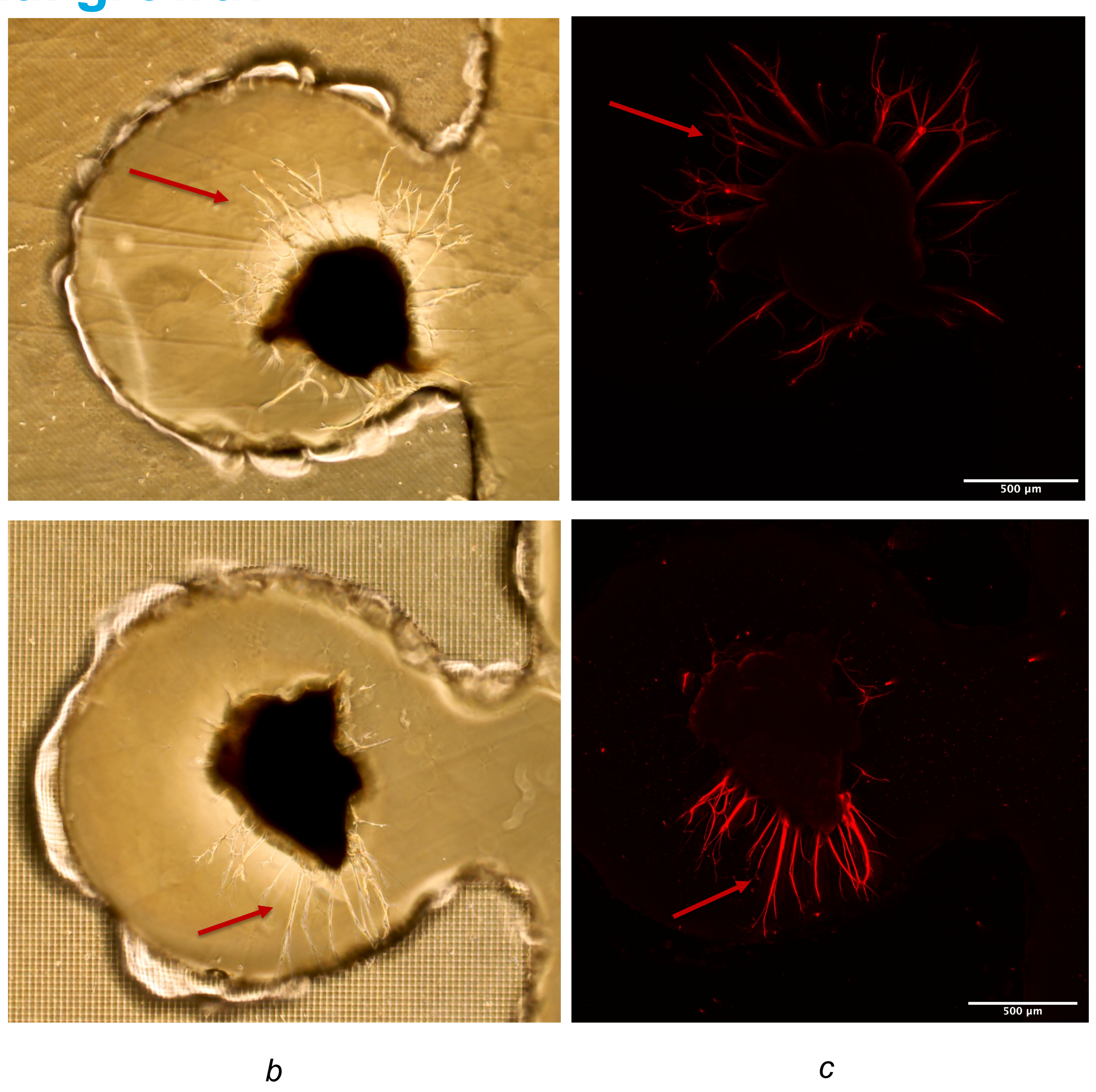


*Figure 2. Cell viability levels of SH SY5Y cells treated with cell culture media extracts from GelMA hydrogels crosslinked using 0.1/1 or 0.3/3 mM Ru/SPS, or normal culture media (untreated group), or sodium dodecyl sulfate (SDS) 10 mM solution (negative control) (**a**); optical microscopy of DRGs growing in 6% GelMA type A 80%DoF hydrogels crosslinked using 0.2/2 mM Ru/SPS via 60s exposure to 450 mW at 50mW (**b**); fluorescent microscopy of DRGs following staining with anti-beta III tubulin, scale bar represents 500 um (**c**).*

Conclusions

- Crosslinking efficiency and density of GelMA hydrogels can be easily tuned through the variations in hydrogel composition parameters.
- DRG demonstrated the growth of neuron extensions in GelMA hydrogels for 2 weeks of 3D cell culture.

References

1. Praxis Spinal Cord Institute. Rick Hansen SCI Registry Community Report. Vancouver, BC: Praxis; 2019.
2. Lim, K. S. et al.. *Macromol. Biosci.* 2019, 19, 1900098.
3. ISO 10993-12. Biological evaluation of medical devices: Section 10.3.3.

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