The University of British Columbia, Faculty of Applied Science and Faculty of Medicine, School of Biomedical Engineering

Visible light-crosslinked GelMA scaffold for neurite outgrowth

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Background

- **A spinal cord injury (SCI) is traumatic and affects every physiological system in the body** (Barbiellini, 2022)
- Symptoms include:
  - Paralysis, pain, bowel and bladder incontinence, and sexual dysfunction
- Over 20 million individuals worldwide live with a SCI, with an incidence of 0.9 million (Safdarian, 2023)
- **Currently no cure on the market**
- Gold standard:
  - Rehabilitative treatments do not effectively promote spinal cord regeneration (Barbosa, 2021) while pharmacological treatments mainly focusing on pain relief (Eller, 2022)
- Hydrogels as a scaffold have been explored to repair damaged neural connections caused by a SCI (Walsh, 2022) but no clinical trials yet
- Gelatin methacrylate (GelMA) hydrogel is fluid and can be precisely transported by a needle into the SCI fluid-filled lesion cavity before being crosslinked into a scaffold using photo-initiators and light (Wang, 2022)
- **Scaffold stiffness influences neurite outgrowth** (Walsh, 2022), **and GelMA stiffness can be tuned** (Young, 2020)

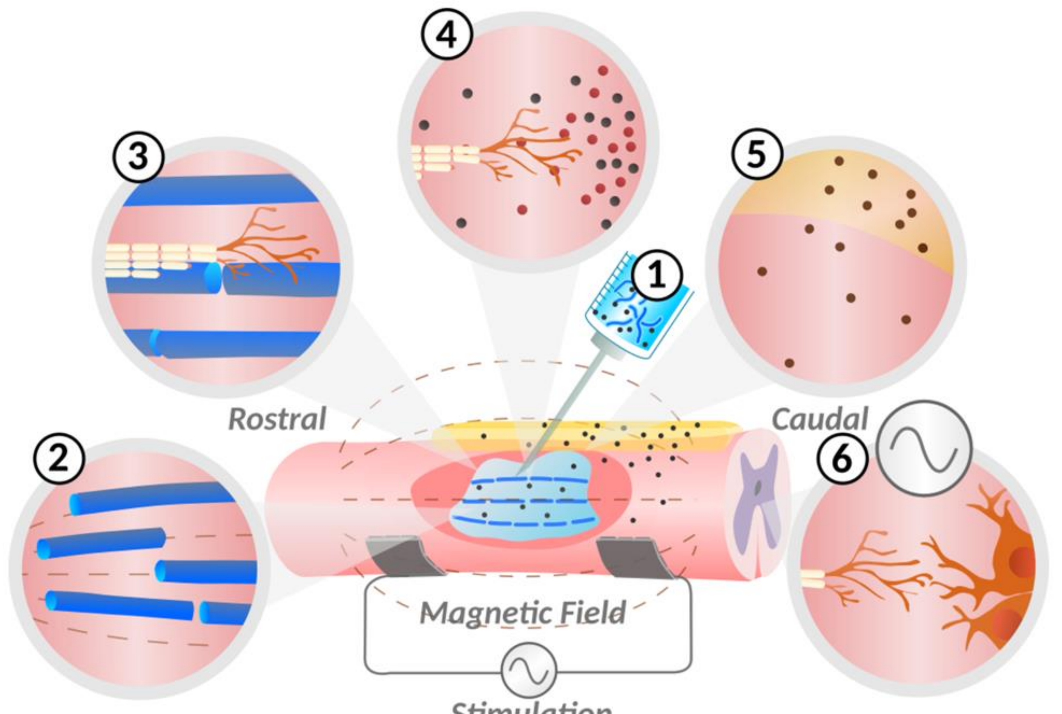


Illustration credit: Mend the Gap

Objectives

I. Fine tune the stiffness of the hydrogel by adjusting the concentration of GelMA or its degree of methacrylation (DoM)

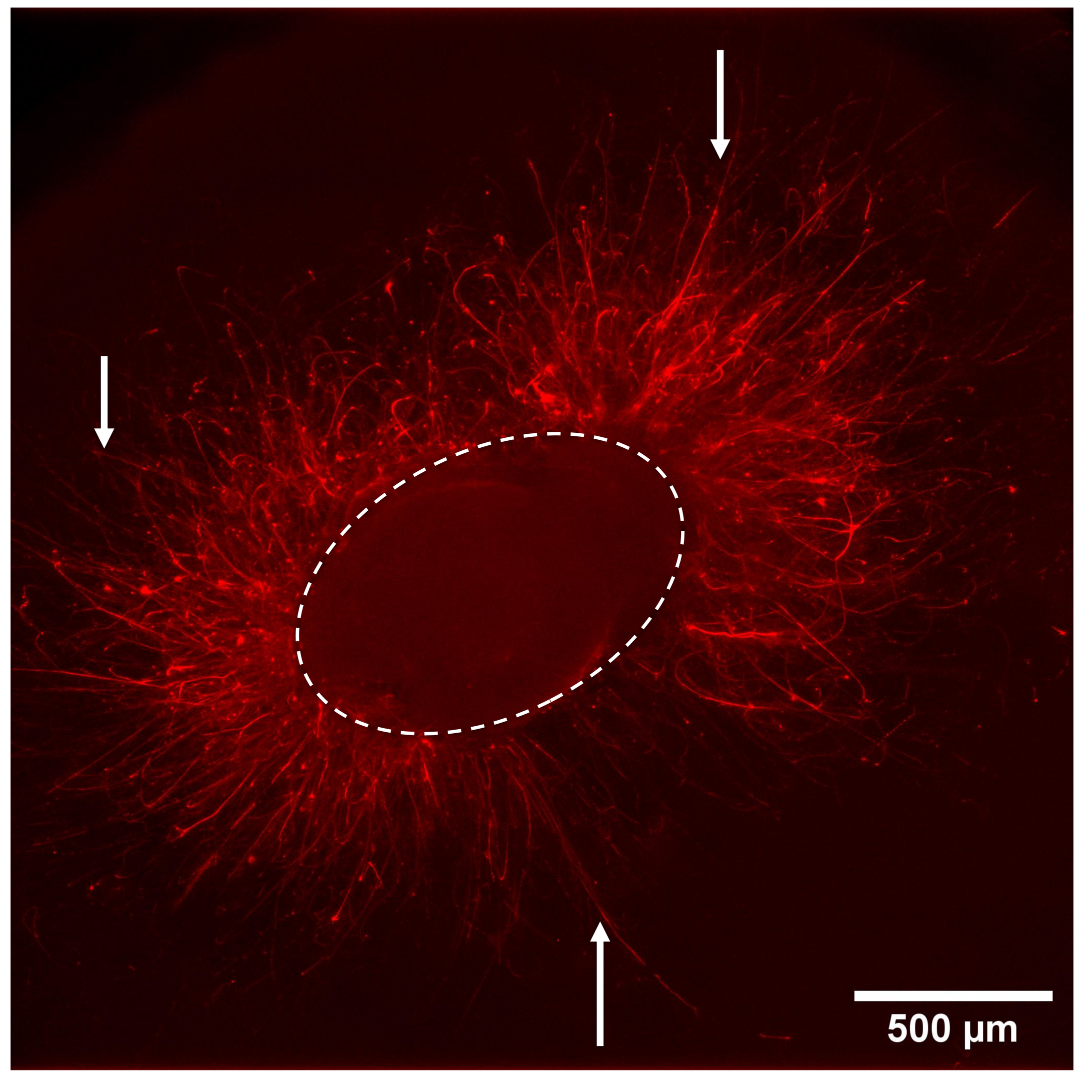
II. Explore if riboflavin and arginine can be used as an alternative photo initiator system to the well-established ruthenium and sodium persulfate

Target stiffness for in vitro neurite outgrowth

Dorsal root ganglion explants are harvested from rat pups and cultured in a spinal cord injury model chip. The control hydrogel is Ultimatrix reduced growth factor (RGF) with a reported stiffness by the manufacturer of 110 Pa. Due to consistent neurite outgrowth in Ultimatrix RGF, ~100 Pa is is the target stiffness for GelMA.

DRG neurite outgrowth in Ultimatrix RGF (control)

Tuj-1 staining of neurites after 7 days of in vitro culture in Ultimatrix RGF. Arrows represent neurites outgrowth while dotted line the contour of the DRG tissue explant.



Rheology

The stiffness of 3% and 6% GelMA and 50% and 80% DoM were assessed

- Photo initiator: 0.2 mM/2 mM Ru/SPS
- Irradiation: 50 mW/cm^2 for 60 s (λ=455 nm)
- Amplitude sweep: 0.1-100 % strain
- Frequency sweep: 0.1-100 Hz
- 25 mm PP/S with solvent trap at 37ºC

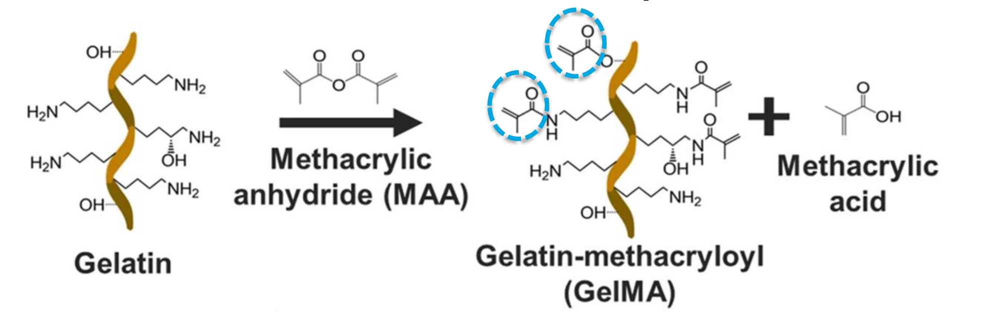
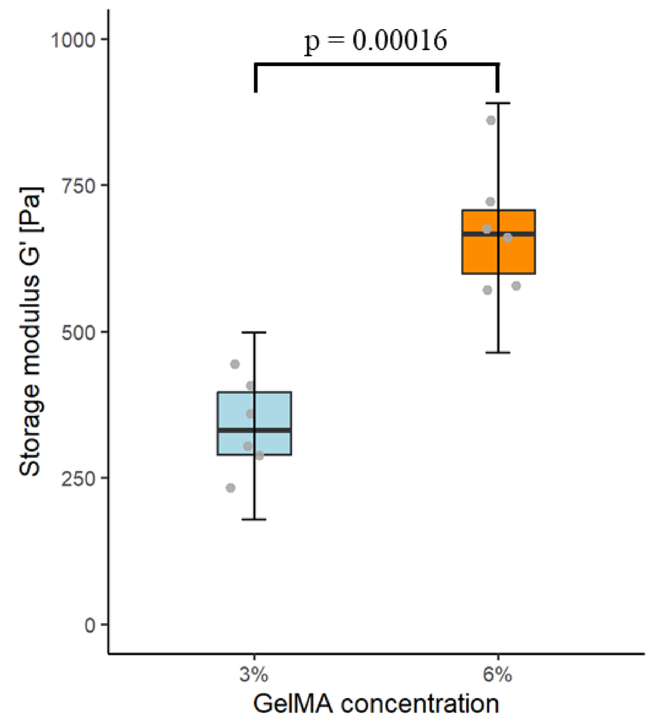


Illustration credit: Shirahama (2016)

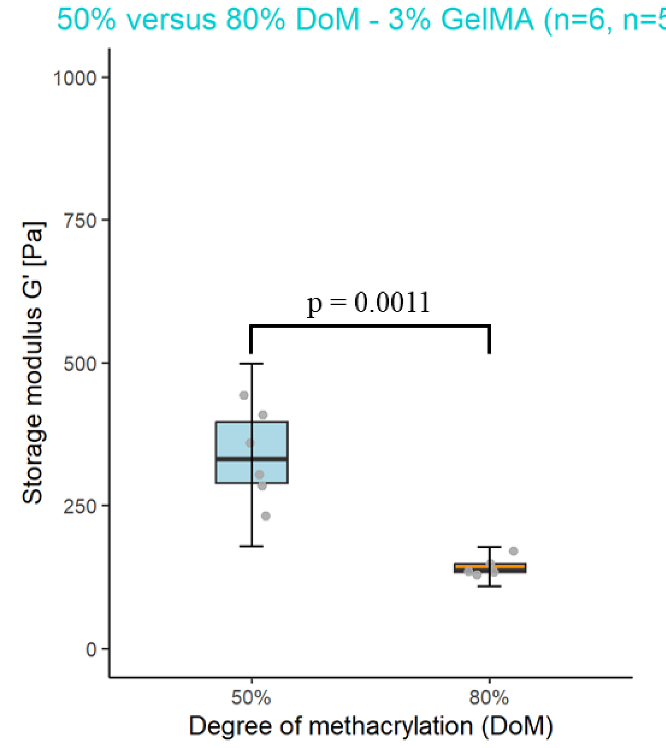
Stiffness versus GelMA concentration and DOM

3% versus 6% GelMA at 50% DoM (top), and 50% versus 80% DoM of 3% GelMA (bottom). Stiffness measured within linear viscoelastic region at 0.4% strain peak-to-peak and 0.5 Hz. Welch's t-test was used for statistical analysis.

3% versus 6% GelMA - 50% DoM (n=6)



50% versus 80% DoM - 3% GelMA (n=6, n=5)

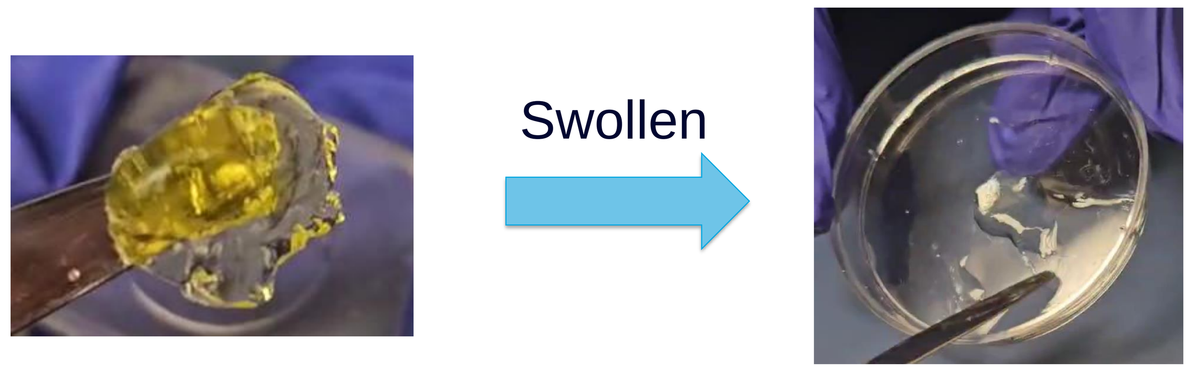


Stiffness of GelMA

The stiffness of 3% and 6% GelMA with 50% DoM is 339 ± 79.7 Pa (n=6) and 677 ± 107 Pa (n=6). Interestingly, increasing the DoM of 3% GelMA to 80% decreased the stiffness to 144 ± 17.3 Pa (n=5) which is close to the target of ~100 Pa. The decrease of stiffness from an increase of DoM is in contradiction to reported findings in the literature which use 10-30% GelMA (Shirahama, 2016; Hoch, 2012; Van Den Bulcke, 2000).

GelMA photo crosslinked with riboflavin, arginine, and PEG-SH

Preliminary data indicates that sodium persulfate (SPS), an oxidizing agent, is cytotoxic to neurons. Whether GelMA photo crosslinked with Ru/SPS is cytotoxic remains to be assessed. As an alternative photo initiator system, riboflavin with arginine (Kim, 2009) and polyethylene glycol thiol (PEG-SH) can crosslink in 60 s at 100 mW/cm^2 (λ=455 nm). The proposed photo initiator system is novel and fully biocompatible.



Conclusion

The hydrogel stiffness was tuned to the target ~100 Pa with 3% GelMA at 80% DoM. A novel riboflavin photo initiator system was explored and could form a hydrogel within 60 seconds.

Acknowledgement

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