Development of Biocompatible Hyaluronic Acid Hydrogel for Nerve Nano-Clip Fabrication

Hossein Rajabzadeh[1,2], Jakob Townsend[2], Morgan Brown[2], Annie Gilbert[3], Tim Gardner[2], Marian Hettiaratchi[2]

[1]Department of Biochemistry, [2]Knight Campus for Accelerating Scientific Impact, [3]Department of Chemistry

Introduction

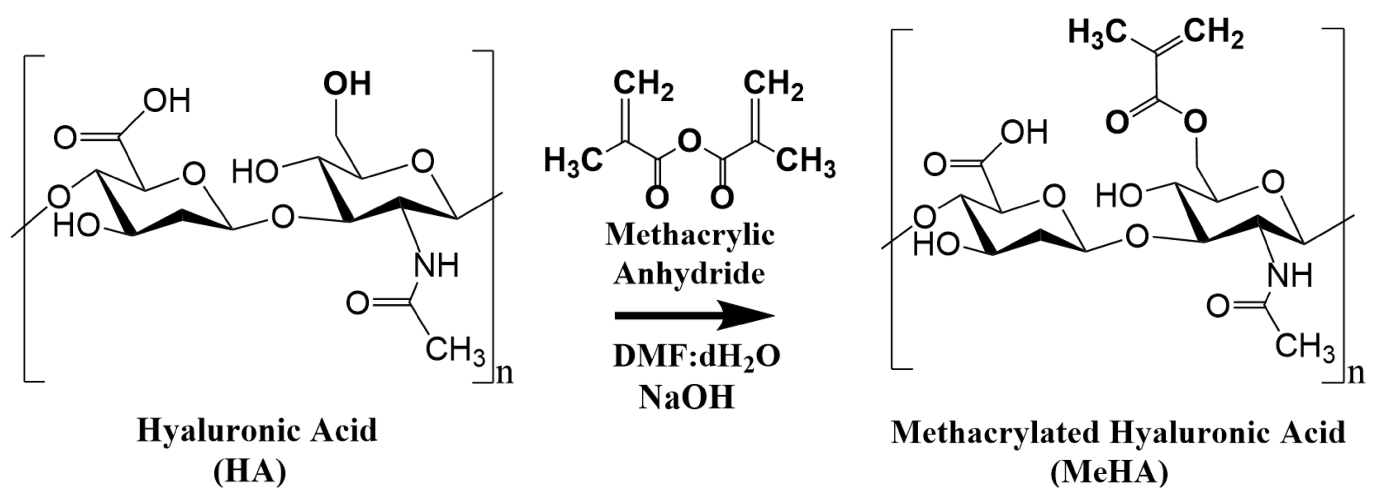
At the University of Oregon, The Gardner Lab used semi-organic Ormocomp and IP-Dip photo-sensitive material to 3D print nano-scale implantable electronic devices called nerve nano-clips. These nerve clips were used to electrically stimulate the tracheosyringeal nerve of Zebra finches and induce addable vocalization[1]. Beyond the studies success, the material composition of the nerve nano-clips also stimulated mild inflammatory response in the surrounding nerve tissue. There is a need for a biocompatible material composition of the nerve nano-clips which can be photo-crosslinked on a nano-scale.

Objective

To developed a biocompatible, minimally-swelling hydrogel from hyaluronic acid (HA) that could be photo-crosslinked for the fabrication of nerve nano-clips that would cause negligible inflammation and nerve tissue damage.

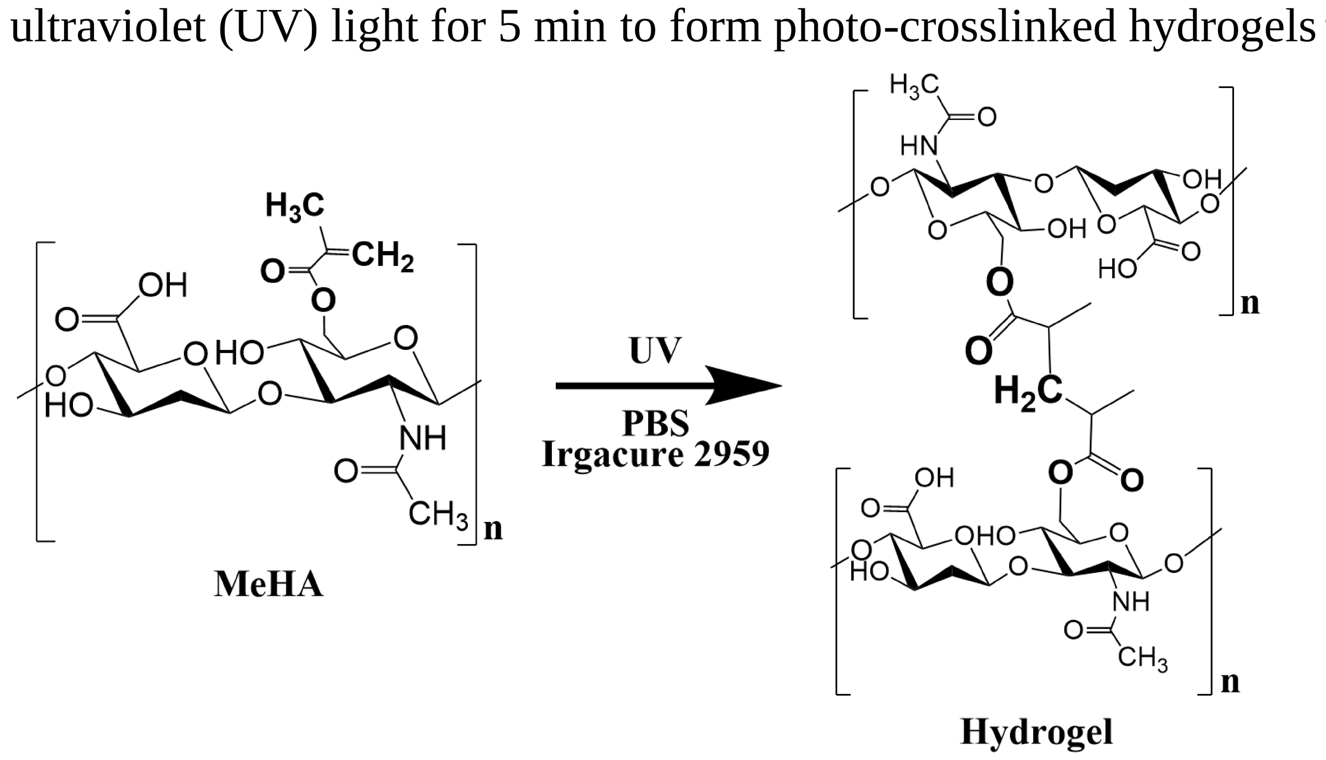
Methods

1) Methacrylated hyaluronic acid (MeHA) was synthesized using an esterification reaction with different molar excesses of methacrylic anhydride (2.5x, 5x, 10x) to HA monomers under basic conditions[2].



2) The degree of methacrylation was quantified with ^{1}H NMR spectroscopy using Brucker AV-500 by comparing the integral peaks at 2.05, 5.78, and 6.21 ppm (HA backbone methyl protons and methacrylate alkene protons, respectively) [3].

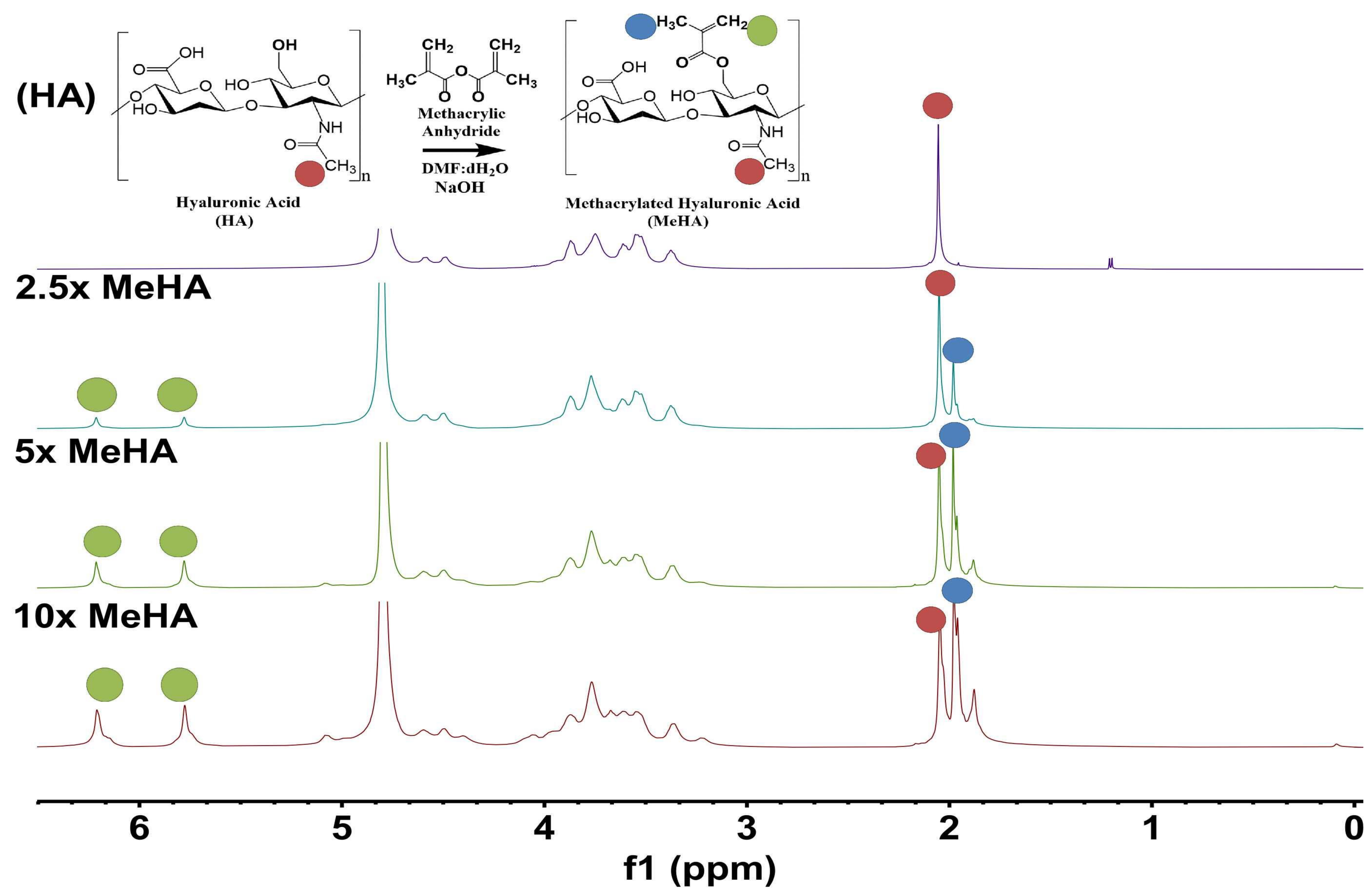
3) MeHA (4% w/v) and a photo-initiator (Irgacure 2959, 10% w/v) were dissolved in phosphate-buffered saline (PBS) and exposed to ultraviolet (UV) light for 5 min to form photo-crosslinked hydrogels [4].



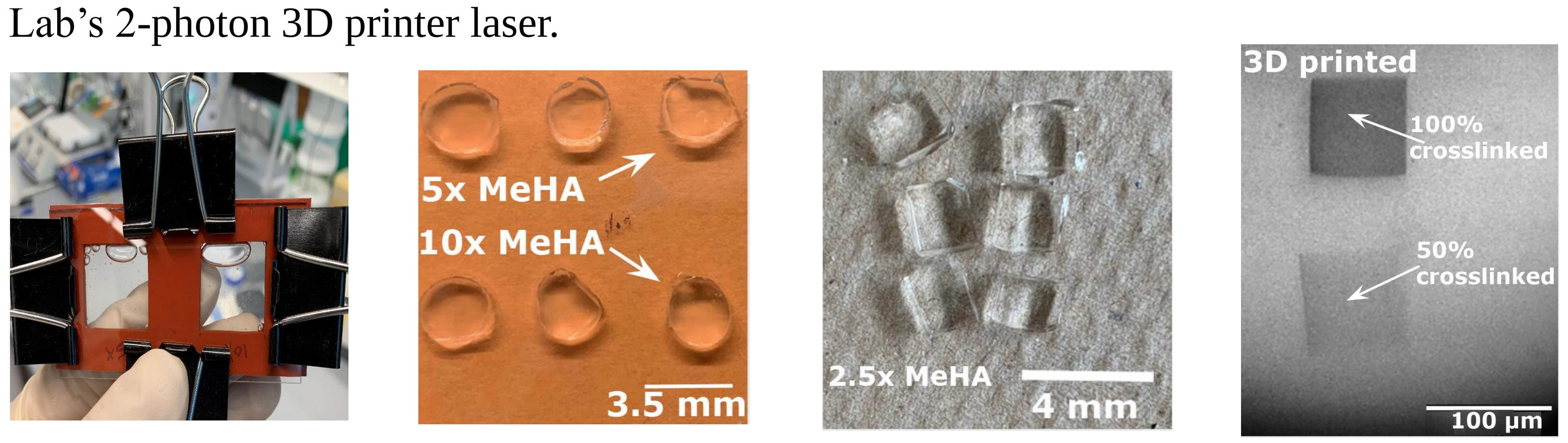
4) MeHA hydrogels were cut into cylinders (radius= 3mm, height= 2mm), placed in PBS, and weighed at initial post-gelation (0h) and increasing time points (4h, 6h, 1d, 2d, 4d, 6d, 8d, 10d). An absorption ratio was calculated by dividing the different time points by the weight at 0h (ideal ratio is 1, which indicates no swelling).

Results

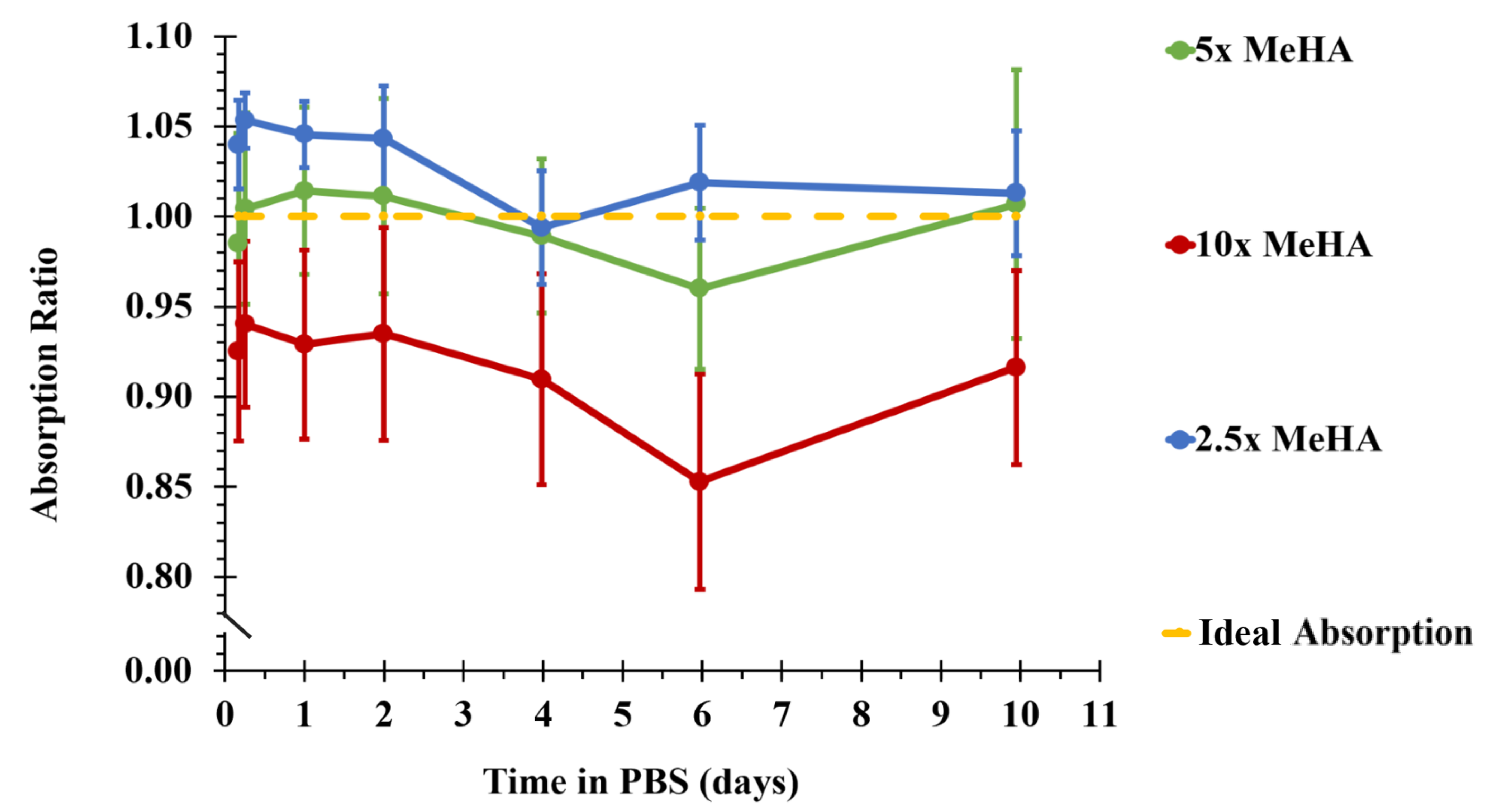
1) Successful synthesis of MeHA conditions were confirmed by ^{1}H NMR spectroscopy due to the appearance of peaks at 5.78 and 6.21ppm (methacrylate alkene proton, green) in 2.5x, 5x, and 10x spectra and their absence in HA spectrum. The transition in peak intensity from 2.05 ppm (red) to 1.98 ppm (blue) pertaining to HA backbone protons and methacrylate methyl alkane protons, respectively, also confirmed MeHA synthesis. ^{1}H NMR spectroscopy revealed the modification levels of 2.5x, 5x, 10x MeHA to be 30%, 46%, and 54%, respectively.



2) MeHA hydrogels (50 µL) were successfully photo-crosslinked in the presence of Irgacure 2959 photo-initiator under UV light. MeHA hydrogels (3 µL) were photo-crosslinked with the Gardner Lab's 2-photon 3D printer laser.



3) The average swelling ratio of 2.5x, 5x, and 10x MeHA over 10 days were calculated to be 1.01, 1.01, and 0.92, respectively. This data suggests that all MeHA conditions swelled under 10%, indicating minimal swelling.



Conclusion

1) Hyaluronic acid biopolymer can be functionalized with methacrylate groups using an esterification reaction under basic conditions to form MeHA.

2) All conditions of MeHA (2.5x, 5x, 10x) were highly photo-sensitive and could be crosslinked in presence of photo-initiator under UV light. MeHA hydrogel solution is a promising candidate for 3D printing complex geometries on a nano-scale.

3) The minimal swelling of MeHA conditions suggest that MeHA could be amendable for use in nerve nano-clip and would support reduced nerve tissue damage.

Future Directions

1) To determine cell viability, neural stem cells could be seeded onto the MeHA hydrogels *in vitro* before implanting the MeHA nerve nano-clips onto the tracheosyringeal nerve of Zebra finches.

2) The conductivity of MeHA could be determined and improved *in vitro* before implanting the nerve nano-clip for electrical stimulation of nerve tissue for *in vivo* studies.

References

1) Otchy, Timothy M., et al. "Printable microscale interfaces for long-term peripheral nerve mapping and precision control." *Nature communications* 11.1 (2020): 1-16.

2) Hachet, Emilie, et al. "Design of biomimetic cell-interactive substrates using hyaluronic acid hydrogels with tunable mechanical properties." *Biomacromolecules* 13.6 (2012): 1818-1827.

3) Spearman, Benjamin S., et al. "Tunable methacrylated hyaluronic acid-based hydrogels as scaffolds for soft tissue engineering applications." *Journal of Biomedical Materials Research Part A* 108.2 (2020): 279-291.

4) Acid H, Kit M, Hydrogels FORU. Directions for Use Lifeink ® 400- Methacrylated Hyaluronic Acid Kit. 2017;1–2.

Acknowledgments

This project received funding from the Hettiaratchi Lab, University of Oregon Undergraduate Research Opportunity Program (UROP) Mini-Grant, and Collins Medical Trust. I would like to thank CAMCOR staff for providing me with the technical support and skills to complete this project. I would also like to thank the Office of the Vice President for Research and Innovations in all work resulting from this research.

Phil and Penny Knight Campus for Accelerating Scientific Impact