

Evaluation of Semi-Interpenetrating Network for Treating Traumatic Optic Neuropathy
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Statement of Purpose: Current treatments for traumatic optic neuropathy (TON) are ineffective at improving visual recovery¹. They do not address secondary neurodegenerative mechanisms such as reactive oxygen species (ROS) accumulation and Wallerian degeneration. To combat these problems, we are designing an in-situ gelling hydrogel scaffold to be placed around the injured nerve site to contract from physiologic stimuli, inducing compressive forces in the nerve to mechanically rejoin the ends of severed nerves. Additionally, methylene blue (MB), a demonstrated neuroprotective and reactive species scavenger^{2,3}, was incorporated within the scaffold to bolster the treatment's therapeutic potential. Here we evaluate a MB loaded, semi-interpenetrating hydrogel network (IPN) for its MB release kinetics and ability to swell from exposure to physiologic ion and temperature conditions.

Methods: Mass ratio blends of alginate (Protanal PH, FMC Corporation, Philadelphia, PA) and xanthan gum (Gelzan, CPKelco, Atlanta, GA) were mixed with MB and evaluated for their material properties, volumetric contraction following calcium chloride-induced gelation, and MB release behavior. Mass ratios evaluated were pure 5 g/L Protanal (Prot), 4:1 Protanal:Gelzan (4P:1G), and 1:4 Protanal:Gelzan (1P:4G). Complex shear modulus was measured using shear-plate oscillatory rheology (Malvern Kinexus ultra+, Malvern Instruments, Worcestershire, UK). Swelling was measured as mass change following hydrogel submersion in 37°C phosphate buffered saline (PBS) with divalent salts. Mixtures of alginate and xanthan gum were crosslinked with 5 g/L CaCl₂ prior to all experiments. Release kinetics of hydrogels loaded with 0.1 g/L MB were characterized by light absorbance at 630 nm of eluent samples using a Synergy H1 microplate reader (BioTek Instruments Inc, Winooski, VT).

Results: Protanal Gels were made with increasing Gelzan content. Addition of Gelzan hastened MB release (**Fig1**) and decreased G* (**Fig2**). Low Gelzan fraction greatly increased the hydrogel's ability to swell (**Fig3**), while a larger content reduced swelling. Pure Protanal, 1P:4G, and 4P:1G hydrogels had complex shear moduli of 3.69±0.22, 1.41±0.04, and 0.15±0.03 kPa respectively (**Fig2**). MB incorporation had minimal effect on hydrogel swelling and modulus (**Figs. 2 and 3**).

Conclusions: Hydrogels made from a combination of alginate and xanthan gum provide a viable means to deliver therapeutics and adequate compression following nerve trauma. Future research aims to include deswelling thermoresponsive hydrogels in degradation and biocompatibility studies.

References: [1] Levin L, et al. (1999). *Ophthalmol*, 106(7), 1268–1277. [2] Talley Watts L, et al. (2014). *J Neurotrauma*, 31(11), 1063–1071. [3] Mei Xiong Z, et al. (2017). *Sci Rep*, 7(1), 1–12.

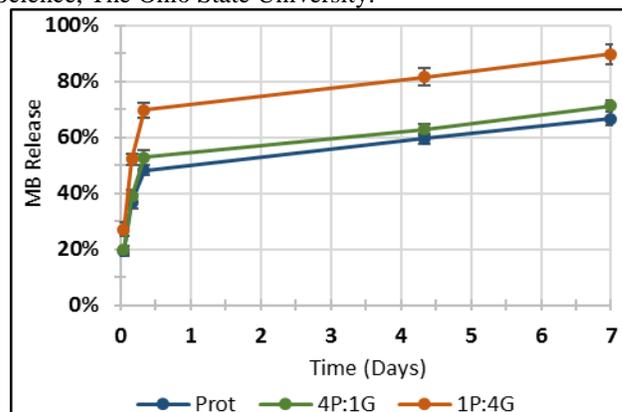


Figure 1. MB release from hydrogels over 1 week. Low Gelzan content increased release magnitude by 4-fold, while a larger content had minimal affect. Each point is the mean ± standard deviation of 4 samples.

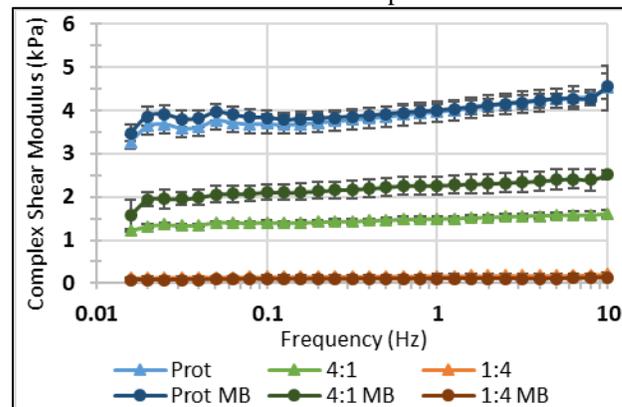


Figure 2. Rheological frequency sweep of hydrogels 0.01-10Hz at 1% strain. G* taken at 0.1 Hz where modulus was independent of frequency. Increasing Gelzan content reduced G*. Each point is the mean ± standard deviation of 3 samples.

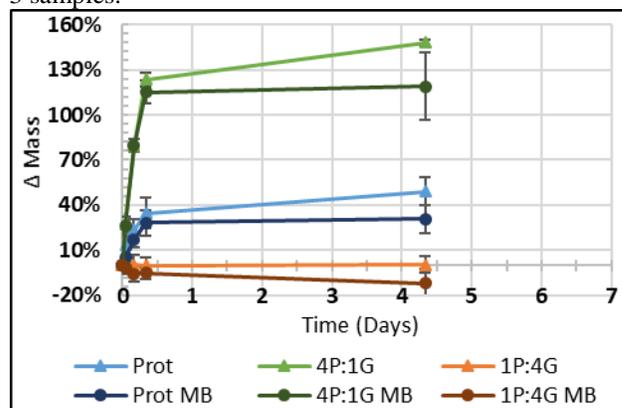


Figure 3. Change in hydrogel mass from water uptake. Small mass fractions of Gelzan increased swelling by 10-fold, while large fractions eliminated swelling. MB incorporation slightly reduced swelling. Each point is mean ± standard deviation of 4 samples.