AWARD NUMBER: W81XWH-15-1-0074

TITLE: Torsion-Induced Traumatic Optic Neuropathy (TITON): Animal Model for Diagnostics, Drug Delivery, and Therapeutics for Injuries to the Central Nervous System

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REPORT DATE: APRIL 2021

TYPE OF REPORT: Annual Report

PREPARED FOR: U.S. Army Medical Research and Materiel Command
Fort Detrick, Maryland 21702-5012

REPORT DOCUMENTATION PAGE

Form Approved
OMB No. 0704-0188

1. REPORT DATE	2. REPORT TYPE	3. DATES COVERED
APRIL 2021	Annual Report	22MAR2020 - 21MAR2021

4. TITLE AND SUBTITLE
Torsion-Induced Traumatic Optic Neuropathy (TITON): Animal Model for Diagnostics, Drug Delivery, and Therapeutics for Injuries to the Central Nervous System

5a. CONTRACT NUMBER

5b. GRANT NUMBER
W81XWH-15-1-0074

5c. PROGRAM ELEMENT NUMBER

6. AUTHOR(S)
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5d. PROJECT NUMBER

5e. TASK NUMBER

5f. WORK UNIT NUMBER

7. PERFORMING ORGANIZATION NAME(S) AND ADDRESS(ES)
Biomedical Engineering
The Ohio State University
2124 Fontana Labs
Columbus, OH 43210

8. PERFORMING ORGANIZATION REPORT NUMBER

9. SPONSORING / MONITORING AGENCY NAME(S) AND ADDRESS(ES)
U.S. Army Medical Research and Development Command
Fort Detrick, Maryland 21702-5012

10. SPONSOR/MONITOR'S ACRONYM(S)

11. SPONSOR/MONITOR'S REPORT NUMBER(S)

12. DISTRIBUTION / AVAILABILITY STATEMENT
Approved for Public Release; Distribution Unlimited

13. SUPPLEMENTARY NOTES

14. ABSTRACT
Deployment-related injuries to the central nervous system (CNS) have increased in frequency since World War II. The increasingly frequent use of improvised explosive devices over the past decade has dramatically increased the rate of these injuries. Unfortunately, rapid diagnosis of these injuries during the window of opportunity for significant CNS regeneration remains problematic. Further, no treatments have yet been developed to the point of clinical applicability which successfully regenerate CNS tissues. We have therefore developed and will use a unique and novel animal model of traumatic optic neuropathy (TON) which allows ready access to the central nervous system for studies on CNS regeneration.

15. SUBJECT TERMS
Nerve injury modeling; traumatic optic neuropathy; diagnosis; neural regeneration

16. SECURITY CLASSIFICATION OF:			17. LIMITATION OF ABSTRACT	18. NUMBER OF PAGES	19a. NAME OF RESPONSIBLE PERSON
a. REPORT	b. ABSTRACT	c. THIS PAGE			USAMRMC
Unclassified	Unclassified	Unclassified	Unclassified	21	19b. TELEPHONE NUMBER *(include area code)*

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1. **INTRODUCTION:** *Narrative that briefly (one paragraph) describes the subject, purpose and scope of the research.*

Traumatic optic neuropathy (TON) is a blinding injury to the optic nerve. While it is known to occur in a significant number of military and civilian injuries each year, no early diagnostic criteria or treatments are currently available. This critical gap is due, at least in part, to the lack of a suitable animal model. We have therefore developed a non-surgical model of TON, as well as several methods which may allow early diagnosis. Phase I of this study will establish those diagnostic criteria, while Phase II will use them to quantify changes in optic nerve structure and function following one of several candidate treatments.

2. KEYWORDS: *Provide a brief list of keywords (limit to 20 words).*

Nerve injury modeling; traumatic optic neuropathy; diagnosis; neural regeneration

3. **ACCOMPLISHMENTS:** *The PI is reminded that the recipient organization is required to obtain prior written approval from the awarding agency grants official whenever there are significant changes in the project or its direction.*

What were the major goals of the project?
List the major goals of the project as stated in the approved SOW. If the application listed milestones/target dates for important activities or phases of the project, identify these dates and show actual completion dates or the percentage of completion.

	Target Date	Completion
Major Task 1: Develop injury diagnostics		
Milestone 1: ACURO approval	June 2019	100%
Milestone 2: Co-author manuscript on diagnostic methods for TON	Sept 2021	60%
Major Task 2: Develop vehicles for delivery of neuroprotective agents		
Milestone #3: Co-author manuscript on hydrogel "cast" for drug delivery and mechanical assistance for neuroprotection	Sept 2021	90%
Major Task 3: Characterization of optic nerve rescue by proposed treatments in vivo		
Milestone #4: Co-author manuscript on changes in optic track following treatment	Mar 2021	0%

What was accomplished under these goals?
For this reporting period describe: 1) major activities; 2) specific objectives; 3) significant results or key outcomes, including major findings, developments, or conclusions (both positive and negative); and/or 4) other achievements. Include a discussion of stated goals not met. Description shall include pertinent data and graphs in sufficient detail to explain any significant results achieved. A succinct description of the methodology used shall be provided. As the project progresses to completion, the emphasis in reporting in this section should shift from reporting activities to reporting accomplishments.

Major Task 1: Develop injury diagnostics
We have completed and updated electrophysiology protocols which will be used to characterize the extent of injury and hopefully serve as a sensitive diagnostic for TON. Briefly, flash electroretinograms (FERGs), flash oscillatory potentials (FOPs), photopic negative responses (PhNRs), and flash visual evoked potentials (FVEPs) will be used to determine the presence, severity, and specific locations of injury. Light adapted (photopic) conditions were selected to ensure sensitivity of the measurement modalities in the absence of dark adaptation. Since many ocular trauma incidents arise with polytrauma, taking time to dark adapt the eye may place an undue (and possibly unnecessary) burden on the patient and medical staff. These protocols were used to evaluate the functional changes in three adult (~400 g) male Sprague-Dawley rats (Charles River Laboratories) following rapid ocular rotation of ~37 degrees. This rotation was selected to cause a low-grade TON to maximize the difficulty in detecting functional changes: it represents a worst-case scenario for diagnostic development.

Methods

Briefly, rats received intraperitoneal injection of ketamine (0.8 mL/kg ketamine; West-Ward; Eatontown, NJ) and xylazine (0.375 mL/kg xylazine; Akorn). Once pain reflex was absent, whiskers were cut back, and the eyes were dilated with 1% Tropicamide. The rat was then placed on a temperature-controlled electrophysiology system (Celeris Testing System with Espion V6 software; Diagnosys LLC; Lowell, MA). A platinum subdermal needle electrode (Natus Manufacturing Limited; Gort, CO. Galway Ireland) was placed in the tail while another was placed at bregma as ground and active electrodes respectfully. One 6 mm gold surface cup electrode (Natus) was placed on top of the tongue to serve as a reference. The eyes of the animal were numbed with 0.5% Tetracaine Hydrochloride Ophthalmic Solution (Bausch & Lomb Inc.; Tampa, FL). The eyes were then lubricated with Systane gel (Alcon Laboratories Inc.; Fort Worth, Texas) or GentleTears gel (Alcon). The eye gel lubricant was then used on the cup of the Diagnosys flash stimulators. The stimulators were aligned in the center of axis of vision and then placed lightly on the corneal surface. FVEPs, FERGs, FOPs, and flickers were obtained in light adapted conditions with a 200 cd.s/m^2 flash. Three PhNR responses were recorded in light adapted conditions using the following stimuli: 200 cd.s/m^2 white flash with a 20 cd.s/m^2 green background, 200 cd.s/m^2 white flash with a 40 cd.s/m^2 green background, and a 200 cd.s/m^2 green flash with a 40 cd.s/m^2 green background. PhNR values were taken from measuring baseline to trough of the resultant wave. After testing was complete, the stimulators were removed from the eyes and cleaned with saline. The electrodes were removed from the animal, and the animal's eyes were cleaned with saline to remove excess eye lubricant. The animal was then injected with an IP dose of Antipamizole (0.4 mL/kg per kg of body weight) to reverse the xylazine and ease the recovery of the animal. This process was utilized to establish baseline values for each animal prior to injury, then repeated 24 hours after injury to determine the diagnostic value of electrophysiology measurements. Finally, measurements were repeated 7 days after injury prior to humane euthanasia and tissue harvesting for histology.

Rats were perfused in phosphate-buffered saline (PBS) followed by 4% paraformaldehyde, and the brain and optic nerve were dissected and incubated overnight at 4°C in 4% paraformaldehyde. Samples were transferred into PBS and shipped to Vanderbilt University Medical Center where the optic nerves and chiasm were dissected away from the brain and cryo-preserved in 20% sucrose in PBS for 2 hrs at 4°C followed by 30% sucrose in PBS overnight at 4°C. The optic nerves were then embedded in OCT (Fisher Scientific, Waltham, MA) and longitudinally sectioned on a Microm HM550 cryostat (Fisher Scientific) at 10 µm thickness. For immunohistochemistry, sections were incubated in PBS to remove the OCT and then incubated in 1:20 normal donkey serum in PBS plus Triton-X-100 (PBT) at room temperature for 2 hrs. Sections were then incubated with specific antibodies as follows: anti-β-tubulin (1:1000; MAB5564; Millipore, Burlington, MA) and anti-glial fibrillary acidic protein (GFAP; 1:50; Z0334; DAKO, Santa Clara, CA) in PBT overnight at 4°C, rinsed with PBS, and incubated in donkey anti-mouse Alexa 488 and donkey anti-rabbit Alexa 594 (1:200; Fisher Scientific) in PBT overnight at 4°C. Finally, the sections were rinsed, mounted in Vectashield plus DAPI (Vector laboratories, Burlingame CA) and imaged on a wide-field fluorescence microscope (Nikon Eclipse, Melville, NY) using consistent settings.

Results

FERG and PhNR responses in the injured eyes of these animals were smaller in magnitude 24 hours and 7 days after injury (Table 1). PhNR, but not FERG, was also decreased in the contralateral eye. FOPs and VEPs remained largely unchanged. PhNR was lowest 24 hours after injury and partially recovered 7 days after injury. Each had smaller amplitude and longer latencies relative to baseline both one and seven days after injury. Immunohistochemical analysis found decreased labeling intensity for both β-tubulin and GFAP in injured nerves relative to sham (Figure 1). Changes

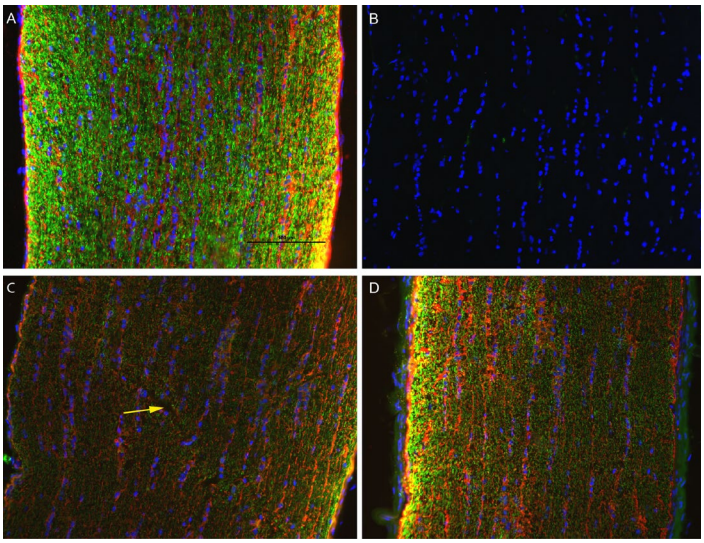


Figure 1: Finite element mesh of the eye and optic nerve. The model is halved on the optic axis to allow visualization of the ocular interior and optic nerve detail.

were observed bilaterally, though not symmetrically, suggesting that the biomechanical mechanism of injury employed must transmit stresses through the optic chiasm.

Table 1: Ocular Rotation and Photopic Negative Responses

			Baseline		D1		D7	
Animal #	**Rotation**	**Maximum Velocity**	**Amplitude**	**Latency**	**Amplitude**	**Latency**	**Amplitude**	**Latency**
	degrees	deg/s	μV	ms	μV	ms	μV	ms
1	42.66	3338	19.8	171	18.9	177	17.3	180
2	37.44	2987	26.9	171	7.6	180	19.3	182
3	32.04	2460	18.3	178	5.2	182	8.7	185
		Mean	21.7	174	10.6	179	15.1	182
		Standard Deviation	4.6	3.6	7.3	2.6	5.6	2.5

Major Task 2: Develop vehicles for delivery of neuroprotective agents

Nine (9) hydrogel formulations were prepared and qualitatively evaluated regarding their ability to form solid, homogeneous hydrogels. Based on this initial screen, an additional 12 formulations were then prepared and characterized for gelation time, swelling behavior, biocompatibility, rheological properties, biodegradation, pH history, and methylene blue (MB) release characteristics. The most promising formulations were then further evaluated to determine the ability of such treatments to maintain cellular viability in an oxidative stress model. Together, these findings form the basis of a manuscript currently in revision with *Drug Delivery and Translational Research*.

Methods

Materials

Sodium alginate (Protanal PH 1033) was provided by FMC Biopolymer (Philadelphia, Pennsylvania). Methylene blue (MB) and Dulbecco's phosphate-buffered saline (DPBS) were purchased from Sigma-Aldrich (Saint Louis, Missouri). Calcium carbonate (CaCO3) was purchased from ChemProducts (Tualatin, Oregon). D-(+)-glucono-1,5-lactone (GDL) was purchased from Alfa Aesar (Haverhill, Massachusetts). Colorimetric 3-(4,5-dimethylthiazol-2-yl)-5-(3-carboxymethoxyphenyl)-2-(4-sulfophenyl)-2H-tetrazolium (MTS) assay and 2,7-dichlorodihydrofluorescein diacetate (DCFH-DA) assay were purchased from Fisher Scientific Inc. (Hampton, NH). Human retinal pigment endothelial cells (ARPE-19, ATCC CRL-2302) were purchased from American Type Culture Collection (ATCC) (Manassas, VA). Dulbecco's Modified Eagle's/Nutrient Mixture F-12 Ham's Medium (DMEM/F-12), phenol-free DMEM, fetal calf serum (FCS), penicillin-streptomycin (PS), trypsin, dimethyl sulfoxide (DMSO) and hydrogen peroxide (H2O2) were all purchased from Thermo Fisher Scientific (Waltham, MA, USA).

Hydrogel Synthesis

Sodium alginate hydrogels were synthesized based on methods reported in literature with modifications. 21 total hydrogel formulations were prepared and evaluated using design of experiments to modify alginate, CaCO3 and GDL concentrations. The first 9 formulations were selected based on

Table 2: Composition of hydrogel formulations.

Formulation ID	Ca^{2+}:Alginate Monomer (mol:mol)	GDL:$CaCO_3$ (mol:mol)	[$CaCO_3$] (g/L)	[GDL] (g/L)
1	0.469	2.493	1.600	7.100
2	0.733	1.596	2.500	7.100
3	0.997	1.173	3.400	7.100
4	0.469	3.301	1.600	9.400
5	0.733	2.113	2.500	9.400
6	0.997	1.553	3.400	9.400
7	0.469	4.109	1.600	11.700
8	0.733	2.630	2.500	11.700
9	0.997	1.933	3.400	11.700
10	0.500	0.125	0.400	0.400
11	0.500	0.250	0.900	0.800
12	0.500	0.500	1.700	1.500
13	0.500	1.000	3.400	3.000
14	1.000	0.125	0.400	0.800
15	1.000	0.250	0.900	1.500
16	1.000	0.500	1.700	3.000
17	1.000	1.000	3.400	6.100
18	1.500	0.125	0.400	1.100
19	1.500	0.250	0.900	2.300
20	1.500	0.500	1.700	4.600
21	1.500	1.000	3.400	9.100

their consistency in forming solid homogeneous hydrogels (Table 2). They served as the models for pH testing in preliminary experiments.

Briefly, sodium alginate (0.63% - 1.85% final w/v in gel) was dissolved in DI H2O by vortexing for 30 seconds and heating in 37°C water for 24 hours. Aqueous 1 mg/mL MB was added to a final concentration of 0.05 mg/mL, followed by the addition of CaCO3 and vortexed. As gelation is initiated rapidly following addition of GDL, the solution was quickly transferred to a mold or onto the rheometer stage following subsequent mixing of all components.

To prepare the remaining hydrogel formulations (10 – 21), GDL:CaCO3 molar ratio concentrations were based off of the original 9 formulations that exhibited a neutral pH of 7.0 ± 1.0 (Figure 2, Table 2). These GDL:CaCO3 ratios ranged from 0.125 – 1.00. The Ca2+:alginate monomer molar concentrations were also evaluated as a factor and varied from 0.5 – 1.5. The ratios were selected to assess the influence of alginate and crosslinker concentrations on drug release, cytotoxicity and viscoelastic properties. Hydrogel formulations 10, 16 and 21 were selected as low, medium and high concentration hydrogels due to their GDL:CaCO3 molar ratios; 0.125, 0.500 and 1.00, respectively. They were analyzed further in cytotoxicity and ROS experimentation.

Hydrogel pH

The pH values of alginate hydrogel formulations 1 – 9 were evaluated using a calibrated pH probe (Mettler Toledo, InLab Expert Pro-ISM, Columbus, OH) for 72 hours to evaluate pH evolution and determine the final compositions of hydrogel formulations 10 – 21. The final pH was reported as the equilibrium pH (pH_E).

Hydrogel Cytotoxicity

The biocompatibility of representative low, medium and high concentration hydrogels (formulations 10, 16, 21) were evaluated using the MTS assay. ARPE-19 cells were first seeded at 5×10^3 cells per well in a 96 well-plate and incubated for 24 hours in 200 µL base media (DMEM/F12, 10% FBS, 1% PS). 1 mL hydrogels were formed in 15 mL conical tubes and allowed to completely gel for 72 hours before 60 – minute UV light exposure, ensuring sterility. The hydrogels were then immersed in 1 mL base media for 24 hours before media collection. The cells were incubated in 200 µL samples for 48 hours prior to performing the MTS assay. A positive control of base media, negative control of 1:9 dimethyl sulfoxide (DMSO): growth media and blank of phenol-free DMEM were used to validate the assay. After incubation, the hydrogel-soaked media was removed and each well washed three times with 200 µL DPBS. Following, 180 µL of phenol-free growth media and 20 µL MTS reagent was added to each well and allowed to incubate for 1 hour. Optical density (OD) of the MTS-treated media was measured at 490 nm using a BioTek Elx808 plate reader (Winooski, VT).

ROS Scavenging

A DCFH-DA assay was used to evaluate the ability of MB to scavenge ROS in cell culture based on the methods of Ludmila et al. (2005) [27]. ARPE-19 cells were seeded on a 96 well-plate at a density of 2×10^4 cells per well in DMEM/F12 media supplemented with 10% FBS and 1% PS and incubated for 24 hours. MB at concentrations of 0 mg/L (positive control), 0.05 mg/L, 0.25 mg/L, 0.50 mg/L, 1.0 mg/L and 2.0 mg/L and a positive control of H_2O_2 and a negative control of DPBS were added to the wells and incubated for 24 hours. Following incubation, the media was removed and 100 µL of DCFH-DA solution was added to each well and incubated for 1 – 2 hours. The cells were washed with DPBS once and the excitation and emission wavelengths; 485 nm and 535 nm, respectively, were measured using a microplate reader.

To further confirm the ROS scavenging ability of MB, 1 mL hydrogels (formulations 10, 16, 21) were formed in 15 mL conical tubes and allowed to gel for 72 hours. Following gelation, the hydrogels were exposed to UV light for one hour to sterilize. The hydrogels were then immersed in 1 mL base media for 24 hours before media collection. ARPE-19 cells were seeded on a 96 well-plate with 2×10^4 cells per well in base growth media and allowed to grow for 24 hours. The culture media was removed and the hydrogel – soaked medium was added to the wells. Hydrogen peroxide (10 µL, 600 µM final concentration) was added to test wells while DPBS was added to the other wells as a negative control. Additionally, hydrogel formulation 16 (medium concentration hydrogel) without MB was included as a negative control. Cells were incubated for 24 hours. Following incubation, the media was removed and 100 µL of DCFH-DA solution was added to each well and incubated for 1 – 2 hours. The cells were thoroughly washed with PBS, and the excitation and emission wavelengths were measured at 485 nm and 535 nm, respectively.

Gelation Kinetics and Mechanical Properties

Oscillatory shear rheology was used to characterize the gelation kinetics, strain amplitude response, and frequency response of alginate hydrogel formulations 10 – 21. The rheometer used was a Malvern Panalytical Kinexus

Ultra+ (Malvern, United Kingdom) with a 20 mm titanium parallel plate upper geometry (PU20 SW1511 TI) and aluminum lower geometry (PLC61 S3722 AL). For all rheological tests, the gap height between the lower and upper geometries, the temperature and sample size were kept constant at 1 mm, 37°C and 375 µL, respectively.

To measure the gelation kinetics of alginate hydrogels, the alginate solution was dispensed as a liquid directly onto the lower geometry of the rheometer immediately following the addition and mixing of GDL. A constant frequency and strain amplitude of 1 Hz and 1% respectively (within linear viscoelastic region), were applied to the sample with its resulting shear stress measured every 5 seconds for 2 hours. The gelation time was defined as the time which gelation had terminated and was determined from the constant frequency and strain test as the first timepoint where complex shear modulus (G*) did not increase by more than 1% of the average of the 10 previously collected measurements. A frequency sweep test immediately followed the gelation test, evaluating the frequency response of the hydrogel. Here, a constant strain amplitude of 1% was applied to the sample while frequency increased from 1 Hz to 100 Hz. The stiffness of the hydrogels is reported as the value of G* at 1 Hz from frequency sweep tests.

Representative low, medium, and high concentration $CaCO_3$ and GDL hydrogels (formulations 10, 16, and 21, respectively) were additionally subjected to an amplitude sweep test to evaluate strain amplitude response. A constant frequency of 1 Hz was applied to the sample while the strain amplitude increased from 0.1% to 100%, and resulting stress was measured.

Hydrogel Swelling and MB Release

1 mL samples of hydrogel formulations 10 – 21 were cast in pre-weighed 15 mL conical tubes and weighed. Formulations 1 – 9 were not included as preliminary studies determined only GDL:$CaCO_3$ ratios of 0.25 – 1.0 were relevant for our studies due to the determined crosslinking maximum between Ca^{2+} ions and alginate. Hydrogels were then immersed in 1 mL DPBS modified without calcium chloride ($CaCl_2$) and magnesium chloride ($MgCl_2$) at 37°C and at regular intervals (0, 1, 3, 7, and 14 days), DPBS was removed and the mass of the hydrogels was recorded. Results were calculated according to the following equation:

$$Q = \frac{M_S - M_D}{M_D} \times 100\%$$

Here, Q is the swelling ratio, M_s is the mass of the formed hydrogel following incubation in DPBS at 37°C and excess water removal and M_D is the mass of the 1 mL alginate solution placed in the tube.

The release kinetics of MB were evaluated using the same formulations (10 – 21) evaluated for swelling. 1 mL hydrogels (10 – 21) loaded with 1 mg/mL MB were created. Following immersion in DPBS and incubation at 37°C, 1 mL DPBS was removed at the given intervals (0, 1, 3, 7, and 14 days). 100 µL samples of the DPBS were placed in a 96 well-plate and absorbance measured. The concentration of MB remaining in hydrogels following DPBS incubation was then determined using a standard concentration-absorbance curve measured at 630 nm using a plate reader (BioTekElx808).

Hydrogel Degradation

1 mL hydrogel solutions based on formulations 10 – 21 were cast in pre-weighed 15 mL conical tubes and weighed. After incubation at 37°C for 72 hours, excess water was removed from tube and hydrogels were weighed again to determine weight following incubation. Hydrogels were immersed in 10 mL 1X DPBS with $MgCl_2$ and $CaCl_2$ at 37°C for 0, 1, 3, 7 or 14 days. At each timepoint, the DPBS was removed, the hydrogels were frozen at -80°C for 24 hours and lyophilized for 24 hours. Hydrogel degradation was reported as the percentage change in the mass of dry components used to create the hydrogel to the dried hydrogel mass after freezing and lyophilization.

Statistical Analysis

Data analysis was performed using two-tailed student t-test. Statistical significance was defined as $p < 0.05$. All values and data points are reported as the average ± standard deviation.

Results

The pH of hydrogel formulations 1 – 9 was recorded for 72 hours (Figure 2A), with all formulations initially at ~6 pH. Formulations demonstrated clear pH value groupings based on GDL:CaCO3 molar ratios. Formulations in the lower group (1, 4, 5, 7, 8) had ratios greater than two, whereas the upper group (2, 3, 6, 9) had ratios less than two. The GDL:CaCO3 molar ratio was plotted against pHE over 72 hours (Figure 2B). Higher variability within the groupings is observed in the lower group with pH values ranging from ~3.5 to ~4.5; however, the variability may be explained by the larger range of GDL:CaCO3 ratios. Greater molar concentration of GDL and CaCO3 caused lower pH. Therefore, formulations with close to neutral pH were evaluated in subsequent studies.

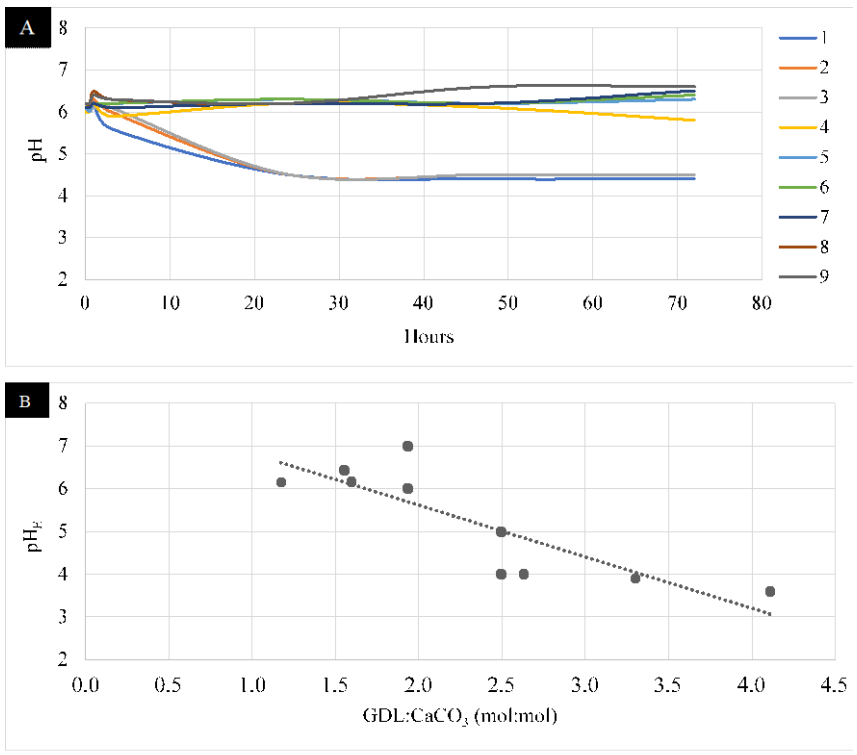


Figure 2: Characterization of pH of hydrogel formulations 1 – 9. (A) Evolution of hydrogel pH over 72 hours. Formulations exhibit clear groupings of pH values. (B) Plot of hydrogel equilibrium pH (pH_E) reached after 72 hours of gelation. There is a linear and inverse relationship between GDL:$CaCO_3$ and pH_E ($R^2 = 0.8$; $p < 0.0001$).

As shown in Table 2, alginate hydrogels 10 – 21 were prepared by varying $CaCO_3$ and GDL concentrations. Time sweep rheology analysis (Figure 4, Table 3) found that different concentrations of the hydrogel components had an observable influence on complex shear modulus (G*). As the concentrations of both $CaCO_3$ and GDL increased, the complex shear modulus also increased. Increasing GDL content significantly increased G* more than the addition of $CaCO_3$. Additionally, complex modulus was dependent on GDL:$CaCO_3$ ratios. Lower ratios corresponded lowered moduli and vice versa. All gelation times for formulations excluding 11 and 14 were significantly different from each other ($p < 0.05$). Gelling time was found to be tunable, decreasing with higher concentrations of both GDL and $CaCO_3$. All hydrogels exhibited a storage modulus significantly greater than their loss moduli and had a G* of at least 35 Pa at 1 Hz.

Table 3: Stiffness and Gelation Time

Formulation	Gelation Time (s)	G* 1 Hz (Pa)
10	2803 ± 40	36 ± 16
11	2025 ± 120	60 ± 21
12	1223 ± 91	125 ± 48
14	2270 ± 26	60 ± 26
15	1517 ± 99	113 ± 53
16	1275 ± 248	247 ± 151
17	707 ± 59	559 ± 34
18	2190 ± 42	70 ± 34
19	1400 ± 72	187 ± 92
20	1055 ± 78	312 ± 134
21	660 ± 198	225 ± 111

The observed influence of the hydrogel composition on G* is detailed in Figure 3. When the Ca^{2+}:alginate and GDL:$CaCO_3$ were 0.500 – 1.000 mol:mol and 0.125 – 0.250 mol:mol respectively, G* gradually increased with time and their respective gelation times were among the lowest (formulations 10, 11, 12, 14, 15, 18), ranging from 1517 – 2803 seconds, or 20 – 48 minutes. When the Ca^{2+}:alginate and GDL:$CaCO_3$ were 1.000 – 1.500 mol:mol and 0.250 – 0.500 mol:mol respectively, G* increased more rapidly with time and their respective gelation times ranged from 1055 – 1400 seconds i.e. 17 – 23 minutes (formulations 16, 19, 20). Lastly, when Ca^{2+}:alginate and GDL:$CaCO_3$ mol concentrations were 0.500 – 1.000 and 0.125 – 0.250 mol:mol respectively, G* dramatically increased within a short period of time and their respective gelation times were among the fastest, averaging around 660 – 707 seconds or 11 minutes to gel completely (formulations 17, 21).

Immediately following the gelation test, a frequency sweep was run on each hydrogel sample in triplicate with the result reported as the average ± standard deviation (n = 3). Figure 4 details the viscoelastic properties of the alginate hydrogels.

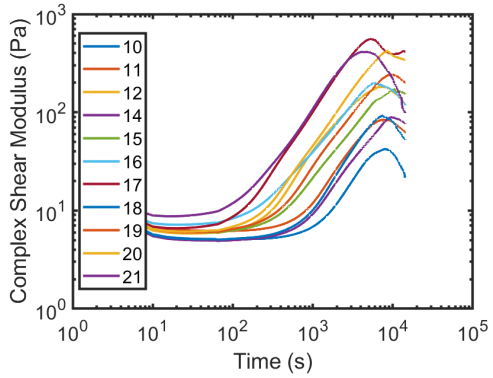


Figure3: Gelation characterization of hydrogel formulations. Time sweep results of hydrogel formulations 10 – 21, excluding 13. Formulations had observable groupings of low and high GDL:CaCO3 ratio hydrogels. Gelation times ranged from 707 ± 59 to 2803 ± 40 seconds.

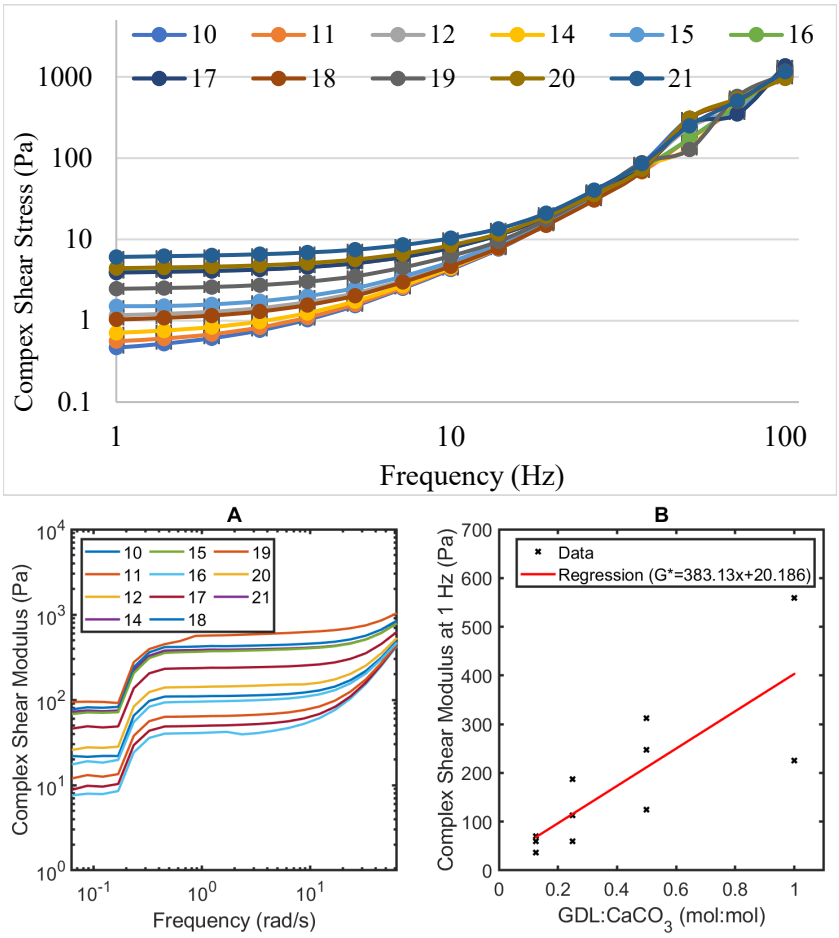


Figure 4. Frequency sweep results of hydrogel formulations. (A) Frequency sweep data from hydrogel formulations 10-21 (except 13). There is a positive exponential relationship between increasing frequency and complex shear stress. (B) Complex shear stress (G*) as a function of concentration ratio ($CaCO_3$:GDL) from hydrogel formulations 10–21 at low frequencies. GDL:$CaCO_3$ ratios significantly influence G*, with higher ratios contributing to high complex shear stresses.

Similar to the grouping for the gelation test, there was grouping observed between low, medium and high concentration alginate hydrogels. The lower crosslinker concentration hydrogels (GDL:$CaCO_3$ ratios of 0.125 and 0.250) corresponded with softer hydrogels and lower complex shear stress whereas higher concentration hydrogels (GDL:$CaCO_3$ ratios of 0.5 and 1.0) were stiffer and therefore had a higher complex shear stresses. The data show that by varying the components of the gels, a significant influence on complex shear modulus and gelation time were observed.

The strain amplitude response of low, medium, and high concentration hydrogels of both $CaCO_3$ and GDL (10, 16, 21) were evaluated via amplitude sweep. Low (formulation 10) and high (formulation 21) concentration hydrogels and medium (16) and high concentration hydrogels were significantly different from each other ($p = 0.0021$ and 0.0006, respectively). All hydrogels contained a linear viscoelastic (LVE) region response to dynamic shear stress, originating at ~1% strain and ending at ~20% (Figure 5). Maximum shear stress varied

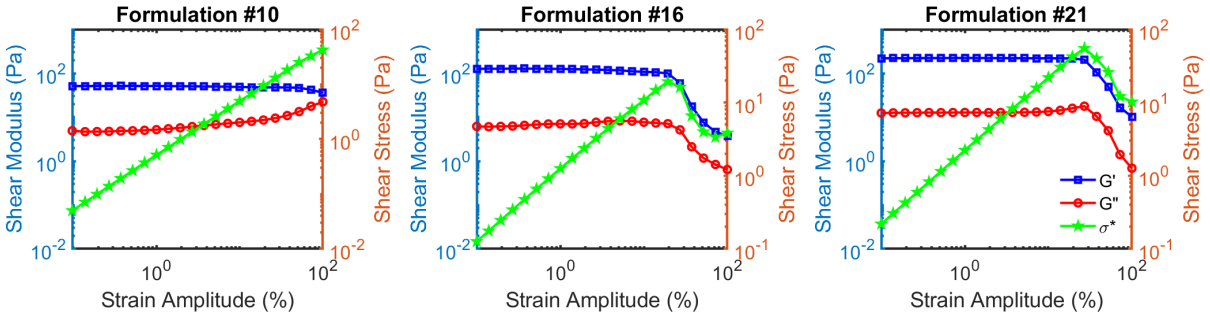


Figure 5. Rheological characterization of hydrogel formulations. Amplitude sweep data from representative hydrogels. A linear viscoelastic region of stiffness response corresponding to 1 Hz dynamic shear is observed up to 1% strain.

among the hydrogels. Formulation 21 demonstrates a sharp increase in complex shear strain from followed by a decrease around 20% complex shear strain, indicative of "fracturing."

Swelling and MB Release of Alginate Hydrogels

The swelling and MB release profile of the hydrogels was recorded *in vitro* over a period of 14 days (Figure 6). The degree of equilibrium swelling varied among hydrogels, ranging from 0 – 150%. Formulations 12 and 21 had the lowest and highest swelling percentage, respectively, correlating to low and high GDL:$CaCO_3$ ratios. The degree of swelling varied is indicative of the components within the hydrogels. Low to medium concentration (of both $CaCO_3$ and GDL) hydrogels had degrees of swelling reported around 100-120%, whereas high concentration hydrogels had swelling above 120%.

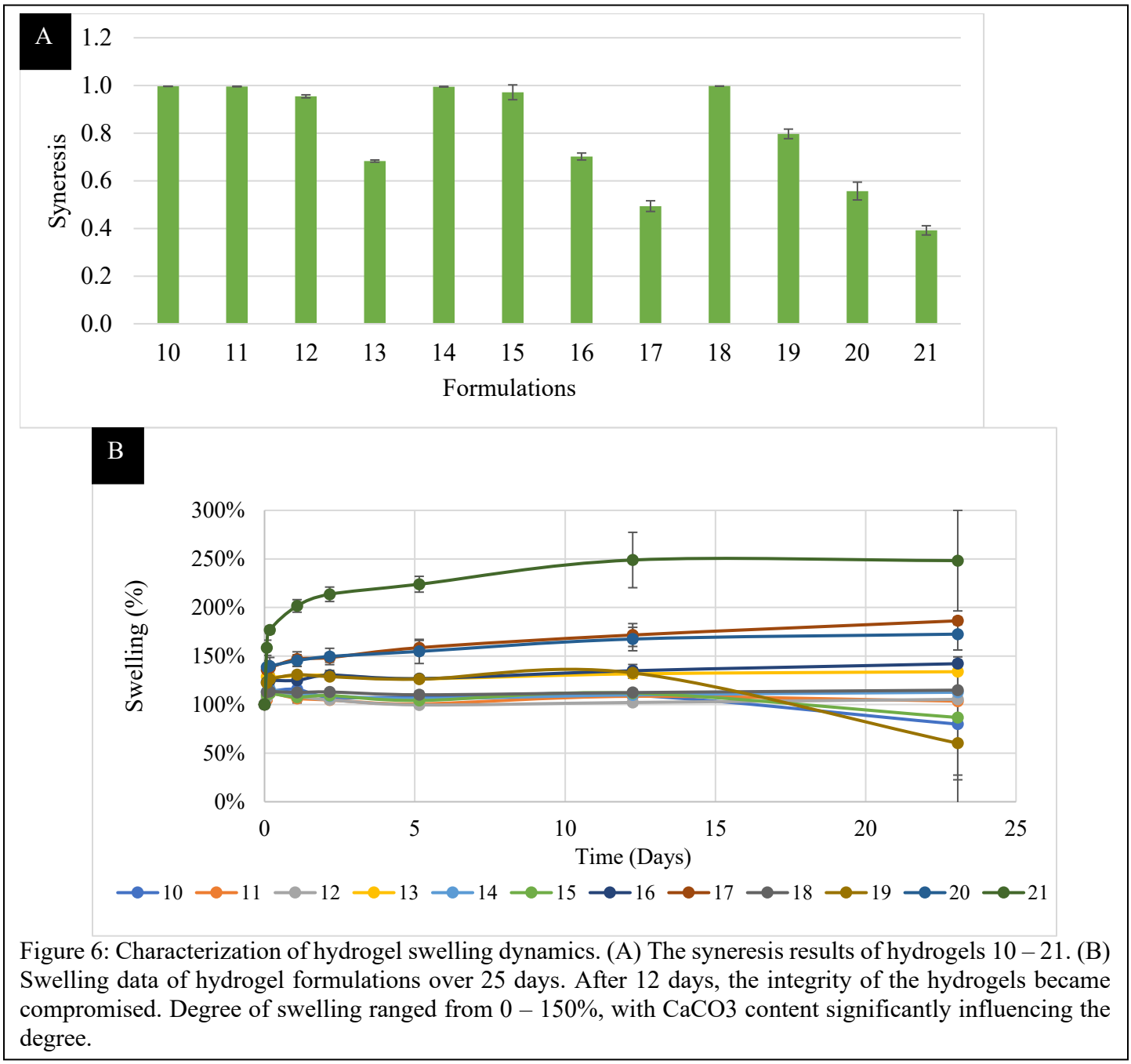
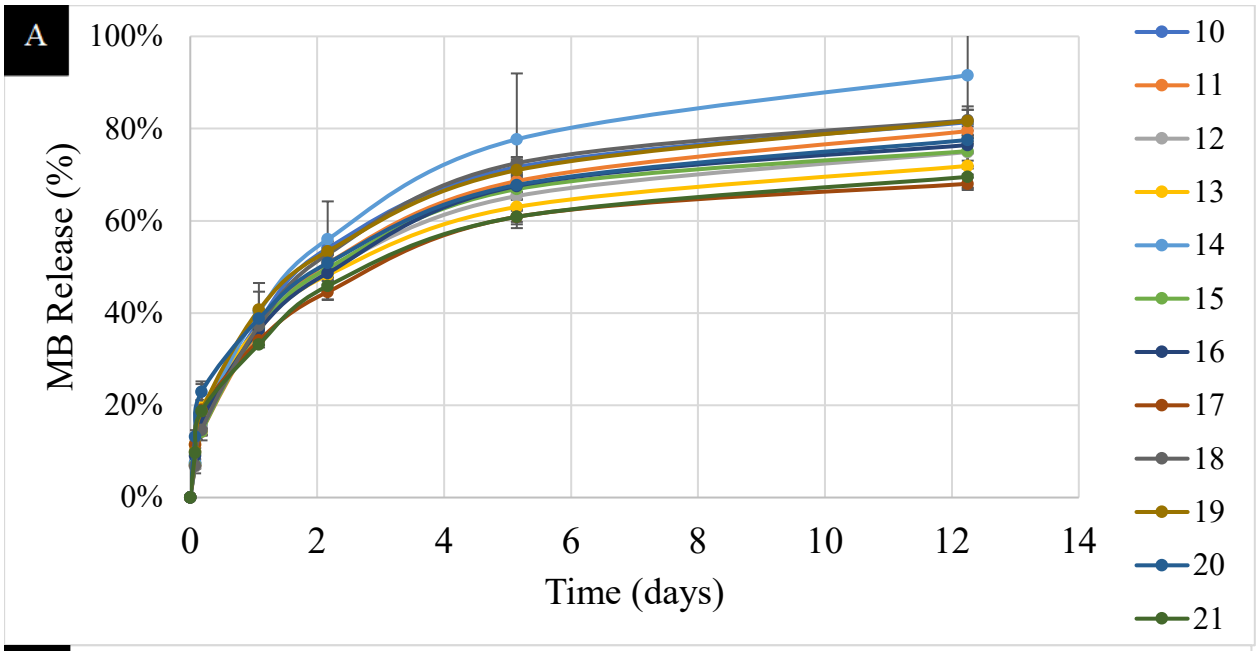


Figure 6: Characterization of hydrogel swelling dynamics. (A) The syneresis results of hydrogels 10 – 21. (B) Swelling data of hydrogel formulations over 25 days. After 12 days, the integrity of the hydrogels became compromised. Degree of swelling ranged from 0 – 150%, with CaCO3 content significantly influencing the degree.

MB release from the hydrogel formulations is further detailed in Figure 7. Among all hydrogels, an initial burst release was observed within the first 5 days, with over 50% MB released. Following the initial burst, a slower and more sustained release followed until the hydrogels disintegrated. Lower concentration hydrogels had the

most cumulative MB release (~90%) by 12 days, the point at which the alginate hydrogels were mostly dissolved and released remaining MB.



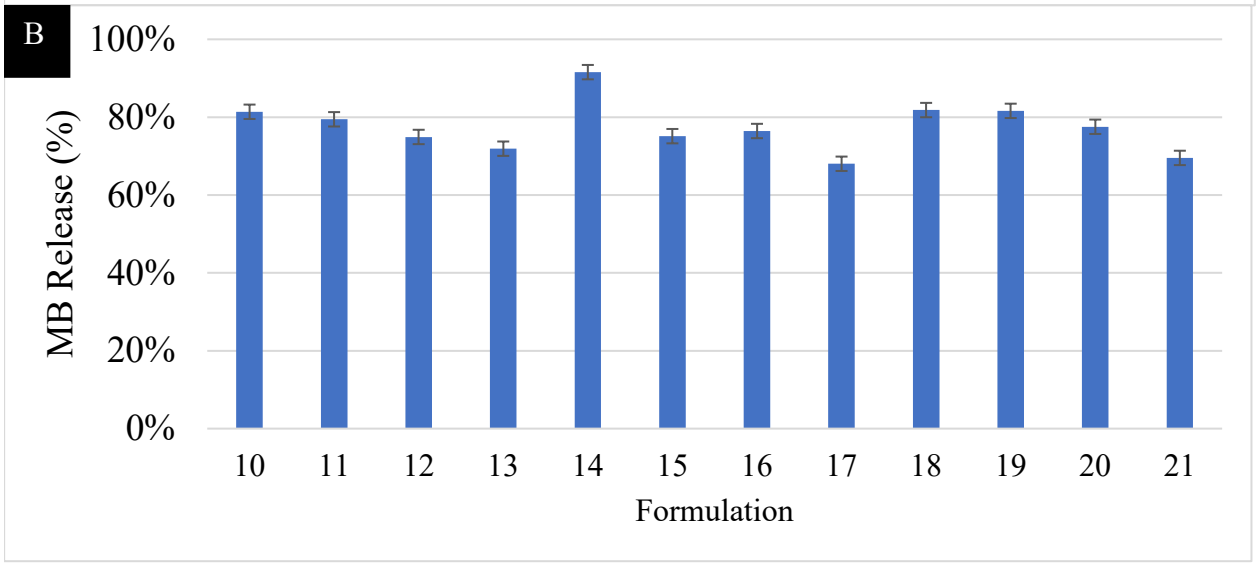


Figure 7: Dynamics of methylene blue release from hydrogels. (A) The release profile of methylene blue of hydrogel formulations 10-21. (B) The cumulative release of MB from hydrogels recorded at Day 12. Medium concentration hydrogels have the highest release profile of low and high concentration hydrogels at each time point.

Cytotoxicity of Alginate Hydrogels

A fundamental requirement for injection is minimal cytotoxicity. To this end, following synthesis of representative hydrogels, we studied their biocompatibility using a human retinal cell line (Figure 8). ARPE-19 cells were incubated with the representative hydrogels for 24 hours and assessed via MTS assay. Formulations 10 and 21, demonstrated excellent cellular viability at over 95%, whereas formulation 16, had viability of 70% of following one-day exposure with the alginate hydrogels. Alginate hydrogels have proven to be biocompatible in various studies and our results using the different crosslinker further support biocompatibility as well as their potential for injection.

MB as a ROS Scavenger

Scavenging of ROS by MB was evaluated through *in vitro* testing based on published methods. ARPE-19 cells were first incubated with MB concentrations of 0, 0.05, 0.25, 0.50, 1.0 and 2.0 mg/mL for 24 hours and then treated with H_2O_2 for 24 hours. ROS levels/activity was characterized by the appearance of highly fluorescent compound DCF in the DCFH-DA assay. There was an observable decrease in fluorescence of the cells corresponding to increased MB concentrations. We confirmed that ROS levels decreased significantly with concentrations of 0.500, 1.00 and 2.00 g/L ($p < 0.05$) (Figure 9). These results suggest the potential of using MB as ROS scavengers for TON treatment.

The ability to scavenge ROS was confirmed with MB (Figure 10). Additional studies were performed with alginate to further confirm MB's ROS scavenging ability while loaded into a hydrogel. All hydrogels were loaded with 1.0 g/L MB, except the negative control 16, which as loaded without MB. ARPE-19 cells were incubated with hydrogel formulations 10, 16, 16 without MB and 21 for 24 hours. Following incubation, the hydrogels and cells were exposed to H_2O_2 for 24 hours with resulting DCF fluorescence measured. Hydrogels 10 and 21 displayed higher degrees of cell survival compared to 16, yielding similar results to our cytotoxicity study. Low and high concentration alginate hydrogels (10, 21) achieved ARPE-19 survival of over 60% when exposed to the highly cytotoxic H_2O_2. Medium concentration hydrogels (16) maintained cell survival of ~35% (with MB); however, survival was lowered to ~10% when cells were exposed to the hydrogels without MB. The presence of MB was found to significantly influence cell survival when loaded into hydrogel formulation 16 ($p < 0.01$) as survival increased from ~10% without MB to 35% with MB.

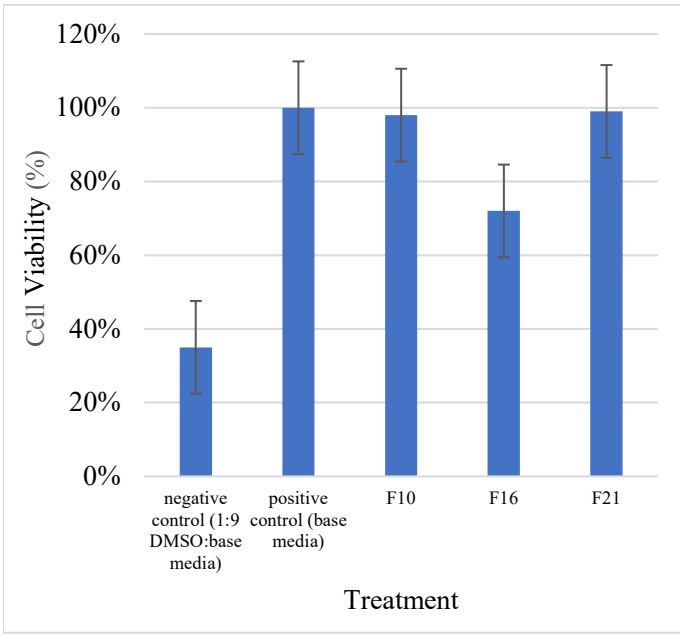


Figure 8: Cytotoxicity of Representative Hydrogels. Cellular viability as measured by optical density (OD) of the MTS reagent product following exposure to alginate hydrogels. The low, medium, and high concentration hydrogels that were evaluated maintained a cell viability of at least 70% that of the positive control base media (DMEM/F12, 10% FBS, 1% PS).

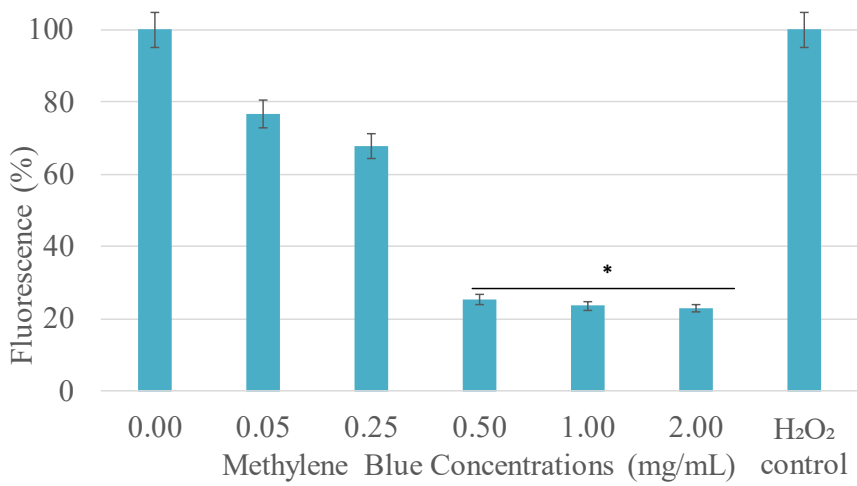


Figure 9: Methylene blue inhibition of oxidative stress. ROS activity measured by DCF fluorescence in ARPE-19 cells induced by 600 µM H2O2. Increased concentrations of methylene blue contributed to higher cell survival during prolonged exposure to H_2O_2. Data ($n = 5$) is presented as mean ± standard deviation. Results were normalized against H2O2 control. Higher fluorescence is indicative of greater DCF presence, more ROS activity and lowered cell survivability. Differences in the fluorescence of MB concentrations of 0.5, 1.0 and 2.0 were found to be statistically significant (*$p < 0.05$).

Naturally derived biomaterials can be advantageous for drug delivery applications as their components can be broken down and removed by the body. Biodegradation of alginate can be more challenging than other biomaterials as it degrades by ion exchange. The *in vitro* degradation of alginate hydrogels was studied for two weeks with the mass of the initial and final mass recorded. Table 4 summarizes the degradation results of hydrogel formulations 10 – 21.

The masses of hydrogel formulations 10 – 21 were recorded over 14 days and weighed at 0, 1, 3, 7, and 14-day timepoints following lyophilization.

Swelling rates varied significantly on Days 0 and 14 based on hydrogel composition. Higher concentration hydrogels displayed the highest degree of swelling on Day 0; however, on Day 14, low concentration hydrogels displayed the highest degree of swelling. The average mass of the hydrogels (mg) following 0, 1, 3, 7, and 14 days were 8.0 ± 1.2, 12.7 ± 1.0, 12.0 ± 0.76, 12.3 ± 2.4, and 18.3 ± 5.4, respectively. On days 1, 3 and 7 of incubation, the degree of swelling as well as the average mass did not differ significantly from previous time points. Low concentration hydrogels among all time points displayed the lowest degree of swelling over time whereas higher concentration hydrogels had the highest degree of swelling, as expected. $CaCO_3$ values were found to influence swelling over time with the lowest GDL:$CaCO_3$ ratio swelling most rapidly. The ratio of Ca^{2+}:alginate was the primary driver of hydrogel swelling with the highest ratios swelling the most overall. Hydrogels prepared with Ca^{2+}:alginate ratio of 0.50 were at approximately equilibrium swelling when formed.

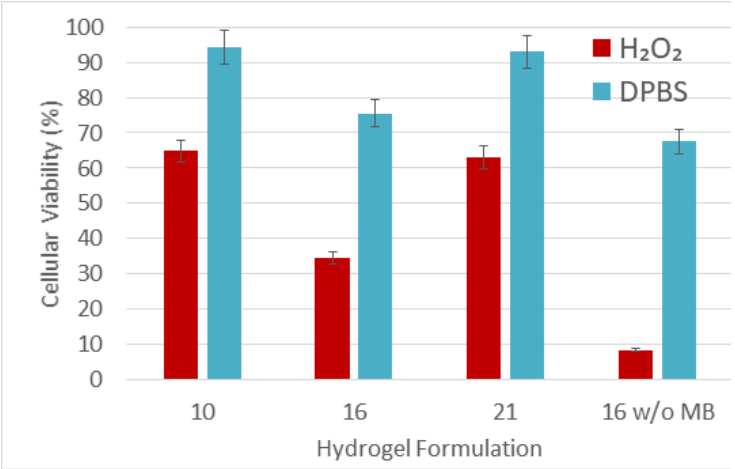


Figure 10: Hydrogel inhibition of oxidative stress. Cell survival was maintained at over 50% for formulations 10 and 21. Cell survival did decrease following exposure to H2O2. Differences between 10 and 21 were found to not be statistically significant ($p > 0.05$). Differences between formulation 16 with and without MB was found

The differences between the hydrogels among timepoints were not significant until Day 14 ($p < 0.05$), with observable groupings between the low, medium and high concentration hydrogels. Low and high concentration hydrogels displayed the highest degrees of swelling in this timepoint (Day 14). Day 0 differed significantly among all timepoints ($p < 0.001$); Day 1 differed significantly from Day 14 ($p = 0.0020$) and Day 7 differed significantly from Day 14 ($p = 0.0021$).

Table 4: Hydrogel Degradation Results

Formulation	Day 0 (mg)	Day 1 (mg)	Day 3 (mg)	Day 7 (mg)	Day 14 (mg)
10	6.4 ± 0.5	12.2 ± 0.2	11.5 ± 0.8	10.3 ± 1.4	24.9 ± 2.2
11	6.4 ± 0.5	13.5 ± 1.0	12.2 ± 0.3	10.5 ± 0.7	24.9 ± 11.0
12	8.0 ± 0.5	12.8 ± 0.5	11.7 ± 0.3	10.6 ± 0.9	13.7 ± 1.4
13	9.6 ± 0.2	14. ± 2.9	12.2 ± 0.8	12.0 ± 0.8	21.3 ± 6.0
14	6.7 ± 0.6	13.8 ± 1.8	13.2 ± 0.3	11.1 ± 0.4	27.7 ± 10.7
15	7.7 ± 0.2	12.6 ± 2.3	11.4 ± 1.1	10.5 ± 0.4	11.8 ± 0.6
16	8.5 ± 0.2	12.3 ± 1.3	11.0 ± 0.1	11.4 ± 0.7	13.8 ± 2.7
17	9.9 ± 0.3	11.4 ± 0.6	11.8 ± 3.2	11.4 ± 0.7	16.1 ± 1.9
18	7.2 ± 0.5	13.7 ± 0.5	12.3 ± 1.1	12.9 ± 7.5	14.7 ± 5.4
19	7.8 ± 0.3	12.8 ± 0.5	11.1 ± 0.9	17.6 ± 3.0	14.0 ± 1.3
20	8.5 ± 0.5	12.9 ± 2.	12.4 ± 2.6	13.4 ± 1.1	15.5 ± 3.6
21	9.3 ± 0.8	10.6 ± 0.9	13.4 ± 3.4	16.3 ± 0.9	20.9 ± 8.8

Major Task 3: Characterization of optic nerve rescue by proposed treatments in vivo

No progress to report.

What opportunities for training and professional development has the project provided?
If the project was not intended to provide training and professional development opportunities or there is nothing significant to report during this reporting period, state "Nothing to Report."

Describe opportunities for training and professional development provided to anyone who worked on the project or anyone who was involved in the activities supported by the project. "Training" activities are those in which individuals with advanced professional skills and experience assist others in attaining greater proficiency. Training activities may include, for example, courses or one-on-one work with a mentor. "Professional development" activities result in increased knowledge or skill in one's area of expertise and may include

workshops, conferences, seminars, study groups, and individual study. Include participation in conferences, workshops, and seminars not listed under major activities.

Graduate Research Assistant Annie Ryan has received extensive training from Dr. Julie Racine. This has included a literature review of visual electrophysiology, including rodent ERG, ERG, VEP, and PhNR; development of protocols relevant for diagnosing TON in our model system; and data analysis procedures. Annie has demonstrated her ability to conduct each aspect of the experiments in rats, producing the preliminary results described above.

How were the results disseminated to communities of interest?

If there is nothing significant to report during this reporting period, state "Nothing to Report."

Describe how the results were disseminated to communities of interest. Include any outreach activities that were undertaken to reach members of communities who are not usually aware of these project activities, for the purpose of enhancing public understanding and increasing interest in learning and careers in science, technology, and the humanities.

Nothing to report.

What do you plan to do during the next reporting period to accomplish the goals?
If this is the final report, state "Nothing to Report."

Describe briefly what you plan to do during the next reporting period to accomplish the goals and objectives.

We are in the process of conducting the experiments required to develop electrophysiological diagnostic markers in Major Task 1. This is essentially iteration of the preliminary results presented above using a larger number of animals. We hope to complete data collection for this Task within the next several months.

We have submitted a manuscript detailing the results of Major Task 2. It is currently in revision.

4. **IMPACT:** *Describe distinctive contributions, major accomplishments, innovations, successes, or any change in practice or behavior that has come about as a result of the project relative to:*

What was the impact on the development of the principal discipline(s) of the project?
If there is nothing significant to report during this reporting period, state "Nothing to Report."

Describe how findings, results, techniques that were developed or extended, or other products from the project made an impact or are likely to make an impact on the base of knowledge, theory, and research in the principal disciplinary field(s) of the project. Summarize using language that an intelligent lay audience can understand (Scientific American style).

Nothing to Report.

What was the impact on other disciplines?
If there is nothing significant to report during this reporting period, state "Nothing to Report."

Describe how the findings, results, or techniques that were developed or improved, or other products from the project made an impact or are likely to make an impact on other disciplines.

Nothing to Report.

What was the impact on technology transfer?

If there is nothing significant to report during this reporting period, state "Nothing to Report."

Describe ways in which the project made an impact, or is likely to make an impact, on commercial technology or public use, including:

- *transfer of results to entities in government or industry;*
- *instances where the research has led to the initiation of a start-up company; or*
- *adoption of new practices.*

Nothing to Report.

What was the impact on society beyond science and technology?
If there is nothing significant to report during this reporting period, state "Nothing to Report."

Describe how results from the project made an impact, or are likely to make an impact, beyond the bounds of science, engineering, and the academic world on areas such as:

- *improving public knowledge, attitudes, skills, and abilities;*
- *changing behavior, practices, decision making, policies (including regulatory policies), or social actions; or*
- *improving social, economic, civic, or environmental conditions.*

Nothing to Report.

5. **CHANGES/PROBLEMS:** *The PD/PI is reminded that the recipient organization is required to obtain prior written approval from the awarding agency grants official whenever there are significant changes in the project or its direction. If not previously reported in writing, provide the following additional information or state, "Nothing to Report," if applicable:*

Changes in approach and reasons for change
Describe any changes in approach during the reporting period and reasons for these changes. Remember that significant changes in objectives and scope require prior approval of the agency.

Nothing to Report.

Actual or anticipated problems or delays and actions or plans to resolve them
Describe problems or delays encountered during the reporting period and actions or plans to resolve them.

Prior setbacks related to grant transfer, ACURO approval, and the COVID-19 pandemic have significantly impacted this project's timeline and budget. We have therefore focused our efforts on Major Tasks 1 developing diagnostic criteria for TON) and 2 (developing a drug-eluting cast for TON). We plan to seek additional funding related to Major Task 3 (determination of safety and efficacy of candidate TON treatments).

Changes that had a significant impact on expenditures
Describe changes during the reporting period that may have had a significant impact on expenditures, for example, delays in hiring staff or favorable developments that enable meeting objectives at less cost than anticipated.

This was in part the result of ACURO's use of an erroneous e-mail address which appears to have been resolved. Contact was re-established in mid-December, requiring only minor comments from our group. While this issue has been resolved, nearly ten months passed before approval was finally granted. We made use of this time to improve training and protocols, as well as perform computational studies, but the personnel expenses incurred during this time were significant and not matched by experimental outputs as agreed in the Statement of Work.

COVID-19 resulted in the sudden closure of all laboratories at Ohio State at a critical time in this project without significantly altering personnel-related expenditures. This loss of time, money, and highly trained personnel have constrained the project's operating budget. We have obtained internal funding for replacement personnel during the training period.

Significant changes in use or care of human subjects, vertebrate animals, biohazards, and/or select agents
Describe significant deviations, unexpected outcomes, or changes in approved protocols for the use or care of human subjects, vertebrate animals, biohazards, and/or select agents during the reporting period. If required, were these changes approved by the applicable institution committee (or equivalent) and reported to the agency? Also specify the applicable Institutional Review Board/Institutional Animal Care and Use Committee approval dates.

Significant changes in use or care of human subjects

No human subjects research will be performed to complete the Statement of Work.

Significant changes in use or care of vertebrate animals

Nothing to report.

Significant changes in use of biohazards and/or select agents

Nothing to report.

6. **PRODUCTS:** *List any products resulting from the project during the reporting period. If there is nothing to report under a particular item, state "Nothing to Report."*

- **Publications, conference papers, and presentations**
  Report only the major publication(s) resulting from the work under this award.

  Journal publications. *List peer-reviewed articles or papers appearing in scientific, technical, or professional journals. Identify for each publication: Author(s); title; journal; volume: year; page numbers; status of publication (published; accepted, awaiting publication; submitted, under review; other); acknowledgement of federal support (yes/no).*

  - Maxwell, C.J., Soltisz, A.M., Rich, W.W., Choi, A., Reilly, M.A., Swindle-Reilly, K.E., Alginate Hydrogels as Injectable Drug Delivery Vehicles for Optic Neuropathy Treatment. *Drug Delivery and Translational Research*, under review.
  - Asemota, B.I., Rodriguez, L., Sponsel, W.E., Ryan, A.K., Racine, J., Rex, T., Glickman, .D., Reilly, M.A., Torsional Indirect Traumatic Optic Neuropathy (TITON): A Physiologically Relevant Animal Model of Traumatic Optic Neuropathy. *Translational Vision Science and Technology*, under review.

  Books or other non-periodical, one-time publications. *Report any book, monograph, dissertation, abstract, or the like published as or in a separate publication, rather than a periodical or series. Include any significant publication in the proceedings of a one-time conference or in the report of a one-time study, commission, or the like. Identify for each one-time publication: author(s); title; editor; title of collection, if applicable; bibliographic information; year; type of publication (e.g., book, thesis or dissertation); status of publication (published; accepted, awaiting publication; submitted, under review; other); acknowledgement of federal support (yes/no).*

  Nothing to Report.

Other publications, conference papers and presentations. *Identify any other publications, conference papers and/or presentations not reported above. Specify the status of the publication as noted above. List presentations made during the last year (international, national, local societies, military meetings, etc.). Use an asterisk (*) if presentation produced a manuscript.*

- Swindle-Reilly, K.E., Maxwell, C.J., Soltisz, A.M., Choi, A., Rich, W., Reilly, M.A., Injectable Alginate Hydrogels for Traumatic Optic Neuropathy, Association for Research in Vision and Ophthalmology, Annual Meeting, Virtual, May 2021.

- **Website(s) or other Internet site(s)**
  List the URL for any Internet site(s) that disseminates the results of the research activities. A short description of each site should be provided. It is not necessary to include the publications already specified above in this section.

  Nothing to Report.

- **Technologies or techniques**
  Identify technologies or techniques that resulted from the research activities. Describe the technologies or techniques were shared.

  Nothing to Report.

- **Inventions, patent applications, and/or licenses**
  Identify inventions, patent applications with date, and/or licenses that have resulted from the research. Submission of this information as part of an interim research performance progress report is not a substitute for any other invention reporting required under the terms and conditions of an award.

  Nothing to Report.

- **Other Products**
  Identify any other reportable outcomes that were developed under this project. Reportable outcomes are defined as a research result that is or relates to a product, scientific advance, or research tool that makes a meaningful contribution toward the understanding, prevention, diagnosis, prognosis, treatment and /or rehabilitation of a disease, injury or condition, or to improve the quality of life. Examples include:
  - *data or databases;*
  - *physical collections;*
  - *audio or video products;*
  - *software;*
  - *models;*
  - *educational aids or curricula;*
  - *instruments or equipment;*
  - *research material (e.g., Germplasm; cell lines, DNA probes, animal models);*
  - *clinical interventions;*
  - *new business creation; and*
  - *other.*

We have developed two novel computational mechanics models relevant to understanding the pathogenesis of traumatic optic neuropathy, as well as evaluation of mechanical aids to treatment. These finite element models allow thorough investigation of biomechanical mechanisms and, when supplemented with experimental data (as described above) may be useful for the study of additional diseases involving the optic nerve (e.g. glaucoma).

- Computational chemo-mechanical finite element model of hydrogel nerve cast

- Computational mechanical finite element model of ocular rotation

7. PARTICIPANTS & OTHER COLLABORATING ORGANIZATIONS

What individuals have worked on the project?

Provide the following information for: (1) PDs/PIs; and (2) each person who has worked at least one person month per year on the project during the reporting period, regardless of the source of compensation (a person month equals approximately 160 hours of effort). If information is unchanged from a previous submission, provide the name only and indicate "no change".

Example:

Name: *Mary Smith*
Project Role: *Graduate Student*
Researcher Identifier (e.g. ORCID ID): *1234567*
Nearest person month worked: *5*

Contribution to Project: *Ms. Smith has performed work in the area of combined error-control and constrained coding.*
Funding Support: *The Ford Foundation (Complete only if the funding support is provided from other than this award.)*

Name: Matthew Reilly
Project Role: PI
Researcher Identifier (e.g. ORCID ID): ORCID 0000-0001-8029-0084
Nearest person month worked: 4
Contribution to Project: Dr. Reilly has constructed mechanical models of ocular rotation and hydrogel casting.

Name: Katelyn Swindle-Reilly
Project Role: co-PI
Researcher Identifier (e.g. ORCID ID): 0000-0003-1739-0263
Nearest person month worked: 3
Contribution to Project: Dr. Swindle-Reilly has coordinated the hydrogel development, has attended meetings related to the research project, and has managed students performing experiments.

Name: Julie Racine
Project Role: co-PI
Researcher Identifier (e.g. ORCID ID): 0000-0003-4409-0936
Nearest person month worked: 2
Contribution to Project: Dr. Racine has developed visual electrophysiology protocols, trained graduate students and employees to implement them, and analyzed electrophysiological data.

Name: Courtney Maxwell
Project Role: Research Assistant
Researcher Identifier (e.g. ORCID ID): N/A
Nearest person month worked: 12
Contribution to Project: Ms. Maxwell has performed experiments with hydrogels, drug release, swelling, pH, rheological and biocompatibility testing. She has been investigating optic nerve biomechanical testing and is first author of a manuscript describing the hydrogel cast.

Name: Danny Mackessy
Project Role: Research Assistant
Researcher Identifier (e.g. ORCID ID): N/A
Nearest person month worked: 4
Contribution to Project: Mr. Mackessy has learned to design and conduct electrophysiology experiments in collaboration with Dr. Racine.

Name: Wade Rich
Project Role: Research Assistant
Researcher Identifier (e.g. ORCID ID): N/A
Nearest person month worked: 12
Contribution to Project: Mr. Rich has been evaluating porcine optic nerve samples for cell separation techniques. He has also contributed to the development of biomechanical testing protocols for the optic nerve.

Name: Bharat Kumar
Project Role: Research Assistant
Researcher Identifier (e.g. ORCID ID): N/A
Nearest person month worked: 4
Contribution to Project: Mr. Kumar has been investigating the most appropriate staining protocols for accurate identification of protein biomarkers.

Name: Annie Ryan
Project Role: Graduate Research Assistant
Researcher Identifier (e.g. ORCID ID): 0000-0003-3645-8649
Nearest person month worked: 7
Contribution to Project: Ms. Ryan has developed electrophysiology protocols and carried out injury modeling in rats.

Has there been a change in the active other support of the PD/PI(s) or senior/key personnel since the last reporting period?
If there is nothing significant to report during this reporting period, state "Nothing to Report."

If the active support has changed for the PD/PI(s) or senior/key personnel, then describe what the change has been. Changes may occur, for example, if a previously active grant has closed and/or if a previously pending grant is now active. Annotate this information so it is clear what has changed from the previous submission. Submission of other support information is not necessary for pending changes or for changes in the level of effort for active support reported previously. The awarding agency may require prior written approval if a change in active other support significantly impacts the effort on the project that is the subject of the project report.

- Nothing to Report.

What other organizations were involved as partners?
If there is nothing significant to report during this reporting period, state "Nothing to Report."

Describe partner organizations – academic institutions, other nonprofits, industrial or commercial firms, state or local governments, schools or school systems, or other organizations (foreign or domestic) – that were involved with the project. Partner organizations may have provided financial or in-kind support, supplied facilities or equipment, collaborated in the research, exchanged personnel, or otherwise contributed.

Provide the following information for each partnership:
Organization Name:

Location of Organization: (if foreign location list country)
Partner's contribution to the project (identify one or more)

- *Financial support;*
- *In-kind support (e.g., partner makes software, computers, equipment, etc., available to project staff);*
- *Facilities (e.g., project staff use the partner's facilities for project activities);*
- *Collaboration (e.g., partner's staff work with project staff on the project);*
- *Personnel exchanges (e.g., project staff and/or partner's staff use each other's facilities, work at each other's site); and*
- *Other.*

Organization Name: Nationwide Children's Hospital
Location of Organization: Columbus, OH
Partner's contribution to the project: Collaboration Dr. Julie Racine, a visual electrophysiologist at Nationwide Children's Hospital, has been instrumental in training personnel for visual electrophysiology measurements and protocol development. She regularly comes to Ohio State for hands-on training and collaborative efforts.

Organization Name: Vanderbilt University
Location of Organization: Nashville, TN
Partner's contribution to the project: Collaboration Dr. Tonia Rex is an associate professor of ophthalmology at Vanderbilt. Her laboratory conducted immunohistochemistry analysis for samples shown in Fig. 1.

8. SPECIAL REPORTING REQUIREMENTS

COLLABORATIVE AWARDS: *For collaborative awards, independent reports are required from BOTH the Initiating Principal Investigator (PI) and the Collaborating/Partnering PI. A duplicative report is acceptable; however, tasks shall be clearly marked with the responsible PI and research site. A report shall be submitted to* ***https://ers.amedd.army.mil*** *for each unique award.*

QUAD CHARTS: *If applicable, the Quad Chart (available on https://www.usamraa.army.mil) should be updated and submitted with attachments.*

9. APPENDICES: *Attach all appendices that contain information that supplements, clarifies or supports the text. Examples include original copies of journal articles, reprints of manuscripts and abstracts, a curriculum vitae, patent applications, study questionnaires, and surveys, etc.*

Torsional Indirect Traumatic Neuropathy (TITON): Animal Model for Diagnostics, Drug Delivery, and Therapeutics for Central Nervous System Injury

MR130235
W81XWH-15-1-0074

PI: Matthew A. Reilly **Org:** The Ohio State University

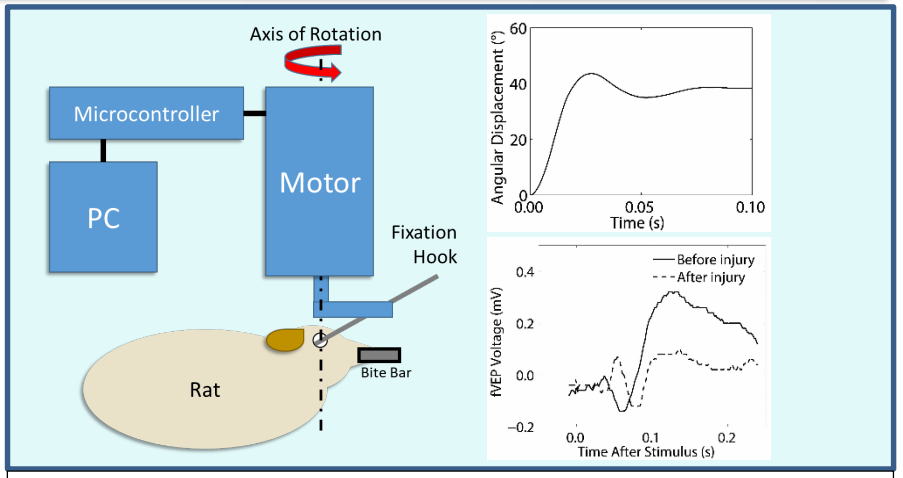
Award Amount: $902,737

Study Aims

- Diagnostics
  - Correlate TITON-induced changes using MRI, VEP, and ERG
  - Identify biomarkers with MALDI-IMS and scRNAseq
- Drug delivery vehicle
  - Develop and characterize drug reservoir hydrogel "cast"
- In vivo evaluation of candidate treatments
  - Treat with one or more therapeutic approach
  - Evaluate post-injury electrophysiology and histopathology

Approach

We have developed a new physiological model of indirect traumatic optic neuropathy (TON). This non-invasive technique achieves injury relevant to blast by rapidly rotating the eye to localize injury near the posterior insertion of the optic nerve. This model offers a simple platform for evaluating diagnostic and therapeutic modalities for TON. We will evaluate new local approaches to treatment including a novel hydrogel "cast" which also serves as a drug delivery reservoir.



Accomplishment: Formulation and characterization of the hydrogel "cast" for drug delivery to the optic nerve is complete.

Timeline and Cost

Activities CY	Lead	2019	2020	2021
Develop injury diagnostics	Reilly			
Develop Tx delivery vehicles	Swindle-Reily			
Evaluate candidate Tx	Reilly			
Estimated Budget ($K)		**$382**	**$423**	**$99**

Updated: 21 June 2021

Goals/Milestones

CY19 Goals – Development of novel materials and methods

☑ Formulate hydrogel cast candidates

☐ Develop diagnostic criteria for TON

CY20 Goal – Treatment studies

☐ Begin in vivo treatment studies in bilaterally injured rats

☐ Characterize treatment efficacy using MRI, DTI, BF

CY21 Goal – Treatment studies

☐ Complete in vivo treatment studies

Comments/Challenges/Issues/Concerns

- The COVID-19 pandemic has significantly impacted the project's timeline and budget.

Budget Expenditure to Date

Projected Expenditure: $902,737

Actual Expenditure: $878,791