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14. ABSTRACT

Penetrating injuries to the eye can lead to decreased intraocular pressure (IOP) and potentially subsequent retinal detachment and loss of vision, if not managed properly. The current standard of care in the civilian setting is to close sclerotomies and other perforations of the sclera are to place sutures which are uncomfortable and can lead to abrasion and infection from eye rubbing. In the theater of operations, the standard is to shield the eye and wait for transportation to a full service base hospital. Here we fabricate and test, both in vitro and in vivo, a sutureless reversibly adhesive wound closure for the eye. The enabling technology is a thermo-reversible adhesive (poly n-isopropyl acrylamide), PNIPAM, which is adhesive to tissues at body temperature and non-adhesive at room temperature. Here we evaluated: 1) PNIPAM sterilization stability, 2) PNIPAM thermal stability, 3) uniaxial adhesion strength to porcine sclera in vitro, and 4) in vitro IOP maintenance in a simulated eye model. Results were compared against cyanoacrylate glue, a common bioadhesive. Lastly, PNIPAM adhesive gel patches were tested in an in vivo (rabbit) model of scleral trauma. The primary endpoint for this study was that the PNIPAM hydrogel patch sustained a greater mean IOP vs. the mean IOP encountered using the current standard of care (no intervention). The secondary outcome evaluated was the tissue response to the PNIPAM material. New Zealand rabbits underwent a surgical sclerotomy to create a full-thickness laceration (3mm in length) through the eye. The treatment group (n=12) received the hydrogel patch and the control group (n=6 received no intervention). IOP measures were taken once every 12hrs for 4 days following the procedure. At each time point, the treatment group exhibited a statistically significant higher IOP vs. the treatment group ($p<0.05$), with the cumulative mean IOP being over 50% greater than the IOP of the control group ($p<0.001$). 30-Day histological evaluation showed no signed of chronic inflammation at the tissue-material interface, suggesting that further biocompatibility assessment should be conducted.

15. SUBJECT TERMS

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1. INTRODUCTION

This project is a preliminary in vitro and in vivo assessment of a novel, tissue repair system for treating penetrating injuries to the wall of the eye (the sclera). The repair system uses a reversibly attachable "patch" on the sclera to temporarily seal a penetrating injury and re-establish the intraocular pressure (IOP) of the eye to afford the casualty a larger window of time before complete surgical intervention can be scheduled. The enabling technology for this system is a thermo-responsive hydrogel based on N-isopropyl acrylamide that is reversibly adhesive as a function of temperature, i.e. it is adhesive at body temperature and non-adhesive below room temperature. The goals of this project were to synthesize a number of different patch hydrogel chemistries and evaluate performance both in vitro and in vivo. In vitro tests included thermoresponsive behavior characterization; characterization of sterilization stability of the hydrogels; characterization of adhesion strength to tissue in vitro, and performance in in vitro models of the eye; and lastly a preliminary performance assessment in an in vivo rabbit model of ocular trauma tracking intraocular pressure and tissue reaction to the hydrogel chemistry. In a one-week implantation study in which full-wall thickness penetrating injuries were created in the scleras of New Zealand rabbits, those that received the hydrogel patch (n=12) yielded an average IOP over 50% greater ($p<0.001$) than rabbits which received the current standard of care, which is no intervention (n=6). A parallel study of ocular tissue response to the hydrogel implanted at the sclera for 30-days showed no significant adverse tissue response at the tissue-hydrogel interface. It is estimated that greater than 10% of combat casualties in the Middle East campaigns suffered ocular injuries[1]. Scleral penetrating injuries cause an immediate loss of IOP in the eye. IOP helps support retinal attachment to the choroidal blood supply of the inner surface of the sclera. If these injuries are left untreated, sustained low IOP increases the risk for retinal detachment which can progress to permanent vision loss [2,3]. This technology would provide a temporary intervention for austere conditions where a casualty may not reach full ocular surgery facilities for several days.

2. KEYWORDS

Ocular trauma; scleral trauma; sclera; hydrogel; PNIPAM; thermo-responsive polymer; open globe; critical care; combat casualty care; poly-n-isopropyl acrylamide.

3. ACCOMPLISHMENTS

a. Major Goals of the Project

SOW Major Goals	Scheduled Period	Actual Period Performed
Fabricate Test Patches	**Q1**	**Q1**
Sterilize Patches	**Q1**	**Q1**
Temperature Exposure of Patches	**Q1**	**Q1**
Adhesion Performance Characterization	**Q3**	**Q3**
Time to Attach/Detach Test	**Q4**	**Q4**
Fabricate Test Patches	**Q5**	**Q5**
Identify Best Test Patches and Fabricate More	**Q5**	**Q5**
In Vivo Sclerotomy Closure Test	**Q5**	**Q07-Q09**
In Vivo Peritomy Test	**Q7**	**Q08-Q10**
Histology	**Q7**	**Q10-Q11**
Review all Data & Summary Report	**Q8**	**Q12**

b. Accomplishments Under Each Goal. (Report Accomplishments)

MILESTONE 1: Fabricate Test Patches

Milestone 1 Major Activities.

In the original proposal, test patches of 100% PNIPAM hydrogel thin films were to be synthesized via Atom Transfer Radical Polymerization (ATRP) on biocompatible substrates (e.g. parylene, polyimide, etc.) [4]. Adhesion data performed on preliminary samples under uniaxial testing showed that the strength of attachment to scleral tissue (porcine) in vitro was significantly lower than cyanoacrylate (a commonly used and FDA approved tissue adhesive for other clinical applications).

Research was refocused on adjusting hydrogel chemistry to improve adhesive performance [5,6]. Different solution chemistries were prepared and compared in a uniaxial tension test to track any performance improvements. Chemistries showing improved adhesion would move on to in vitro IOP testing.

Milestone 1 Specific Objectives.

- Synthesize supported hydrogel patches.
- Characterize adhesion strength to sclera and compare performance to cyanoacrylate.
- Synthesize unsupported hydrogels using two different block co-polymer compositions, and a range of different hydration concentrations.
- Identify which chemistry/ies exhibit the best adhesion characteristics.

Milestone 1 Significant Results & Key Outcomes

Key Outcome 1: Three types of hydrogel polymer chemistries were prepared via ATRP synthesis technique: 1) PNIPAM monomer, 2) copolymer of NIPAM with n-tert butylacrylamide, and 3) copolymer of NIPAM with butylacrylate. *Table 1.1* shows the chemistries that were prepared along with additional characteristics and properties.

Table 1.1 Co-Polymers Tested

	PINPAM	PNIPAM:N-tert Butylacrylamide (N_xT_y)	PNIPAM:Butylacrylate (N_xBA_y)
Chemical Formulae:	$(C_6H_{11}NO)_x$	$(C_6H_{11}NO)_x$:$(C_7H_{13}NO)_y$	$(C_6H_{11}NO)_x$:$(C_7H_{12}O_2)_y$
Co-Polymer Ratios Tested:	N/A	(85:15)	(95:5); (88:12)
Avg. Molecular Weights:	2.864×10^5	5.55×10^5 to 6.624×10^5	3.00×10^4
Percent Aqueous Solution Concentrations Tested:	10%, 14.2%, 25%, 30%, 43.2%	10%, 15%, 20%, 30%	10%, 20%, 30%
LCST (^oC):	32	25	14-16

Key Outcome 2: The original proposed patch design, using the original PNIPAM chemistry did not exhibit adhesion properties on par with a comparable existing adhesive (cyanoacrylate).

Different PNIPAM patches were prepared by varying aqueous hydration from 0.8% to 43.2% hydration. When tested in uniaxial tension, it was found that the adhesion increased with increasing hydration. However, when compared to cyanoacrylate tested in the same linear pull test, it was found that 43% aqueous PNIPAM exhibited only 80% of the adhesion strength of cyanoacrylate to scleral tissue.

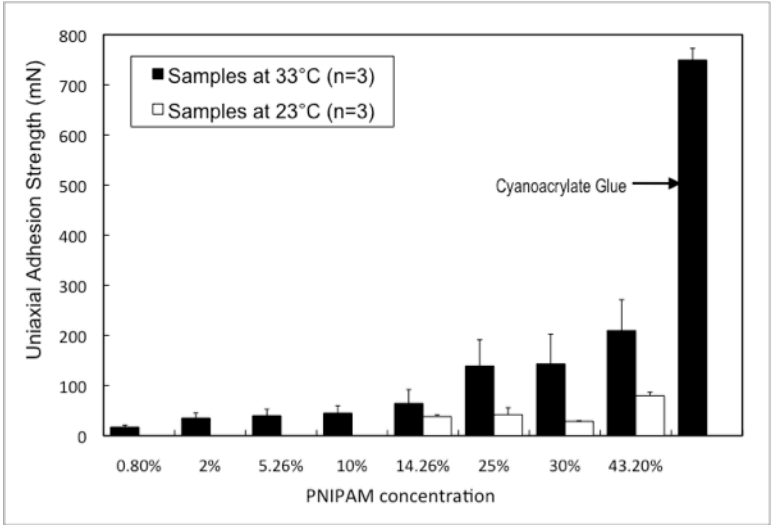


Figure 1.1 Uniaxial adhesion strengths of PNIPAM of different % hydration at room (white) and body (black) temperatures, compared to cyanoacrylate

When tested using the Intraocular Pressure Test Model, the 43% PNIPAM did not meet success criteria (complete arrest of saline infusion at 16mm Hg pressure). The 43% PNIPAM, was tested in the IOP test protocol to measure ability to arrest leakage under ocular pressure conditions. Infusion rates for pre-incision, post-incision creation, post-suturing and post patch placement are shown in *Table1.2*. While leak rates were lower than any other sample tested to date, these results still show leakage, and therefore we cannot begin to initiate in vivo testing.

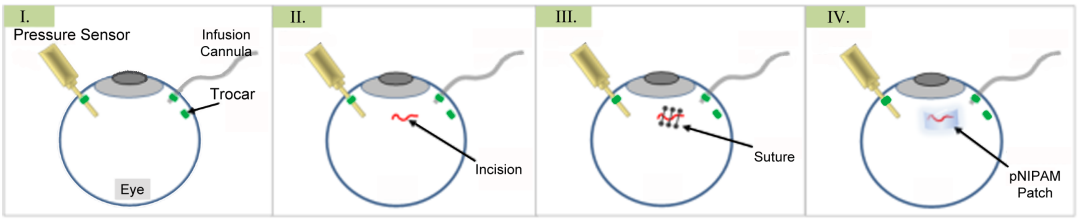


Figure 1.2 Schematic showing four stages of the IOP test.

Table 1.2 Saline Infusion Rates in Porcine Eye Model of Scleral Penetration				
	Infusion Flow Rate (cc/min)			
Adhesive Material Tested	**I.**	**II.**	**III.**	**IV.**
Cyanoacrylate Glue	0	18	0	0
43.2% PNIPAM at 16.5 mm Hg	0	19	0	12
10% (85% PNIPAM: 15% n-tert ButylA) 10mm Hg	0	10	0	**5**
10% (85% PNIPAM: 15% n-tert ButylA) 20mm Hg	0	13	0	**7.5**

Key Outcome 3: Co-polymer composition hydrogels used in an unsupported (injectable gel) format exhibited better adhesive performance vs pure PNIPAM hydrogel in the in vitro IOP test model. It was discovered that injecting the hydrogel at the penetration in the form of a free-standing hydrogel resulting in an effective occlusion of the scleral penetrations. Tests evaluating PNIPAM co-polymerized with butylacrylate showed consistently higher pressure values with most IOP measured at 77mm Hg. It was decided that further efforts would focus on PNIPAM-butylacrylate compositions.

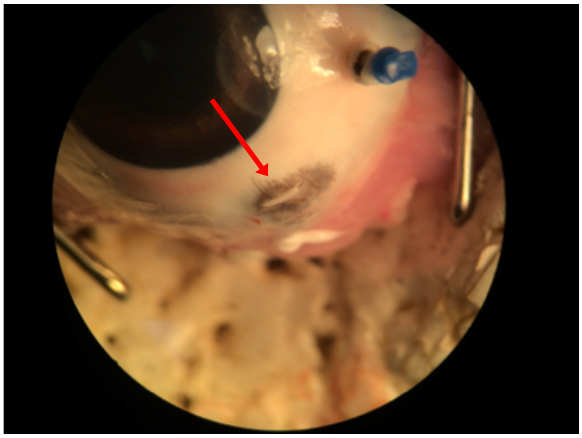


Figure 1.3 Optical micrograph of porcine eye with 3mm incision which has been sealed with copolymer hydrogel (red arrow) and maintained pressure after infusion with saline.

Key Outcome 4: The porcine eye model showed some inconsistencies in data recording. Repeated testing using the whole eye IOP test model showed wide ranges in variation.

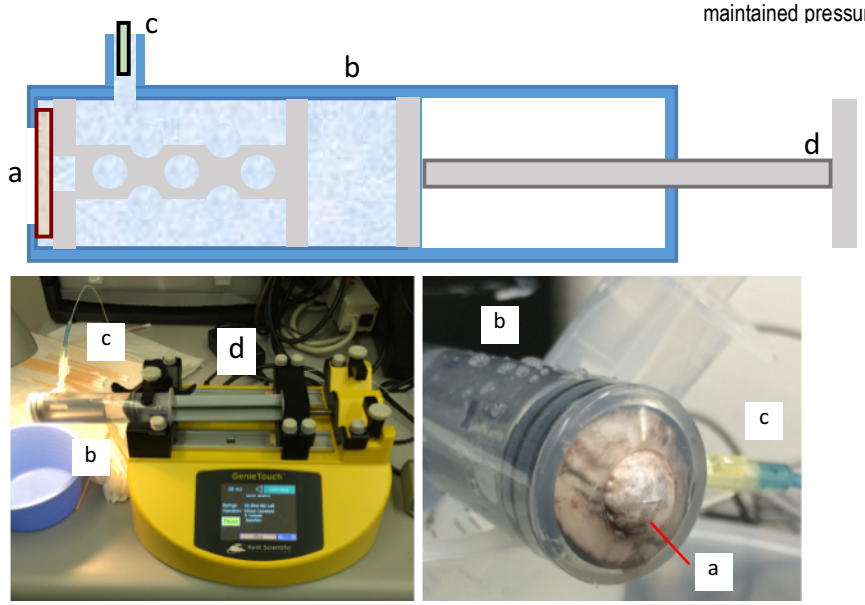


Figure 1.4 Cross-sectional schematic (top) and associated photographs (bottom) of revised IOP test system in which hydrogel samples are tested on a dissected section of scleral tissue. Dissected sclera(a) is mounted into a modified 60mL syringe (b) with a custom port (c) for filling with saline and insertion of the pressure sensor. Pressure is controlled using a digitally controlled infusion system connected to the syringe plunger (d).

Differences in the amount of vitreous removed, and the location of the vitreous with respect to the rest of the test setup (e.g. pressure sensor location, infusion cannula location) led to wide variance in observed IOP. It was decided that a more controllable IOP test system should be designed to reduce variations from test to test.

Milestone 1 Other Achievements

A digitally controlled model for in vitro IOP testing was developed (previous page Figures) using a digital infusion system coupled with a modified syringe with coupled pressure sensor to simulate the eye. A section of dissected porcine sclera is used at the modified aperture to serve as the test surface. Limiting the tissue to only a patch of dissected scleral reduced variations that were seen and likely caused by differences in connective tissue and vitreous in the whole eyes.

MILESTONE 2: Patch Sterilization

Milestone 2 Major Activities

PNIPAM chemistries were prepared and subsequently sterilized via ethylene oxide gas (ETO) exposure and autoclave. PNIPAM reversible transition between adhesive and non-adhesive states is associated with transitions between hydrophobic and hydrophilic states. Contact angle measurements (associated with hydrophobicity/philicity) as a function of temperature were performed on hydrogel adhesives both pre-sterilization and post-sterilization. Also FTIR measurements were performed pre- and post-sterilization to look for changes in polymer chemistry bonds that might indicate degradation. Changes in contact angle measures or FTIR measures were used as indicators of sterilization causing irreversible changes to the polymer chemistries.

Milestone 2 Specific Objectives

- Prepare hydrogel samples via Atomic Transfer Radical Polymerization (ATRP) and Chemical Vapor Deposition (CVD)
- Perform baseline contact angle measurements and FTIR scans
- Subject samples to ETO sterilization or autoclave sterilization
- Perform post-sterilization contact angle measurements and FTIR scans

Milestone 2 Significant Results & Key Outcomes

Key Outcome 1: PNIPAM is unaffected by ETO sterilization, independent of synthesis route (ATRP vs. CVD). Both FTIR and autoclave test results showed a minimal change in temperature dependence of these properties pre- and post-ETO sterilization. A standard ETO sterilization protocol that exposed samples to vaporized ethylene oxide gas at 55°C at a concentration between 400-800mg/L for 3 hours was used. Minor differences in measurements between pre- and post-sterilization were within the standard error.

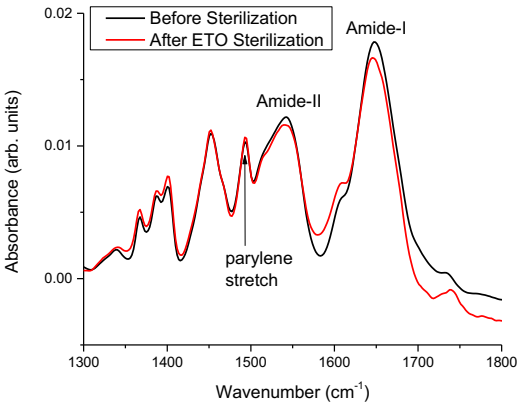


Figure 2.1. Top row: FTIR spectra recorded for PNIPAM hydrogel pre- and post-ETO sterilization. Bottom row: Contact angle vs. temp measurements pre-sterilization (blue) and post-sterilization (red), for (A) ATRP-synthesized PNIPAM on parylene patches, and (B) CVD-synthesized PNIPAM on parylene patches.

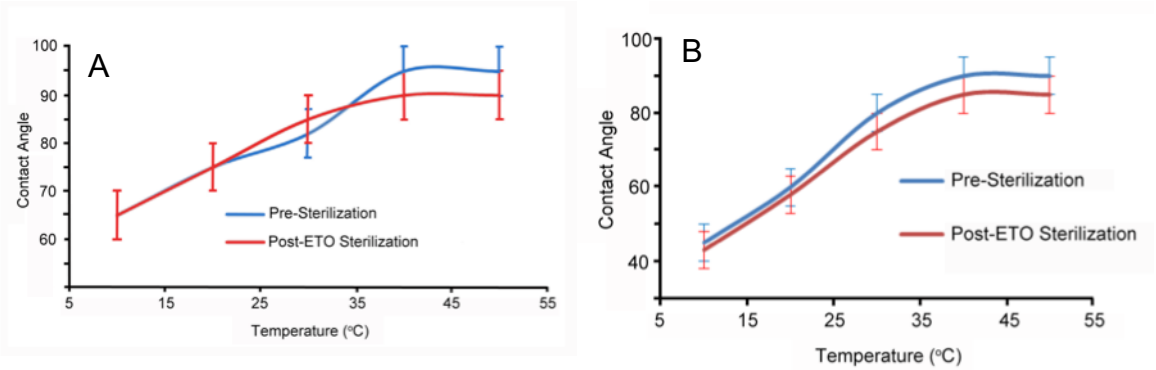


Figure 2.2. Top row: FTIR spectra recorded for PNIPAM hydrogel pre- and post-ETO sterilization. Bottom row: Contact angle vs. temp measurements pre-sterilization (blue) and post-sterilization (red), for (A) ATRP-synthesized PNIPAM on parylene patches, and (B) CVD-synthesized PNIPAM on parylene patches.

Key Outcome 2. PNIPAM hydrogel temperature dependence is unaffected by autoclave sterilization. The autoclave sterilization protocol exposed samples to steam heated to between 130°C for 15 minutes followed by cooling. Here again, we see that the temperature dependence of the contact angle was minimally affected by the temperature exposure.

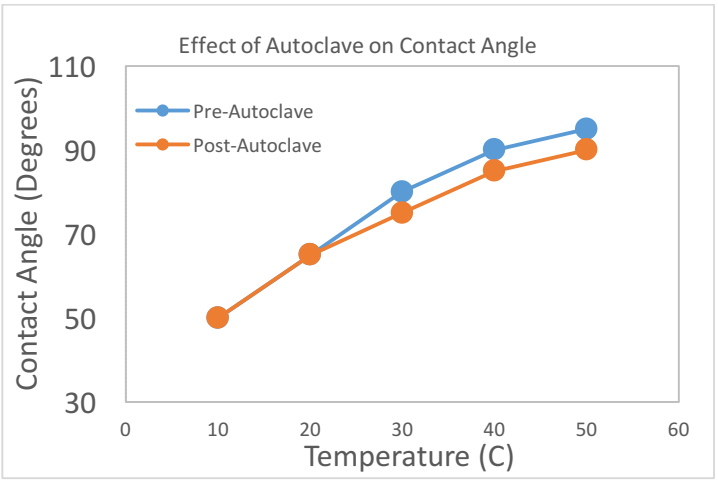


Figure 2.3. Comparison of PNIPAM hydrogel contact angle v. temperature performance pre- and post-autoclave sterilization.

Milestone 2 Other Achievements
None to report.

MILESTONE 3: Temperature Exposure of Patches

Milestone 3 Major Activities

A temperature exposure protocol was developed based on the MIL-STD-810G guidance for high temperature and low temperature exposure [7]. These protocols (described below) were designed based on input from advisors and literature, and are used to provide a preliminary assessment of high temperature and low temperature exposure during transportation. Criteria for success was that the pre- and post-exposure contact angle measures showed less than a 10% difference.

High Temperature Protocol Key Characteristics*

- Duration: 3 Day
- Thermal protocol: 24hr thermal cycling
- Temperature Range: 90F to 120F
- Test samples are placed inside simulated packaging
- Over chamber for 72hrs

Low Temperature Protocol Key Characteristics*

- Duration: 3 Day
- Thermal protocol: Constant Temperature
- Temperature Exposure: -46F [Cold (C2)]
- Test samples are placed inside simulated packaging
- Freezer storage for 72hrs.

Milestone 3 Specific Objectives

- Fabricate samples
- Run temperature exposure protocols
- Conduct post-exposure contact angle measurements

Milestone 3 Significant Results & Key Outcomes

Key Outcome 1: Table 3.1 lists the summary results of contact angle data results on the three different hydrogel chemsitries that were tested. In summary, the average change in contact angle measures pre- and post- temperature exposure for all chemistries was less than a 10% difference. This was consistent with expectations as these hydrogel polymers are known to be temperature stable.

Table 3.1	**High Temperature Protocol**	**Low Temperature Protocol**
PINPAM	Pass	Pass
PNIPAM:N-tert Butylacrylamide (N_xT_y)	Pass	Pass
PNIPAM:Butylacrylate (N_xBA_y)	Pass	Pass

Based on these result we feel reasonably comfortable that hydrogel chemistries should survive most packaging and transportation challenges encountered in a deployment setting.

Milestone 3 Other Achievements

None to repot.

MILESTONE 4: Adhesion Performance Characterization

Milestone 4 Major Activities

The hydrogel patch design pivoted to development of an unsupported, injectable hydrogel adhesive gel (vs. the original patch design). The mode of function, illustrated in *Figure 4.1*, would be to create a plugging occlusion to seal the eye, and would leverage the ability of the hydrogel to conform to any size. As a result of this change, a second series of IOP tests were performed using the automated in vitro IOP test (infusion pump system described in Milestone 1).

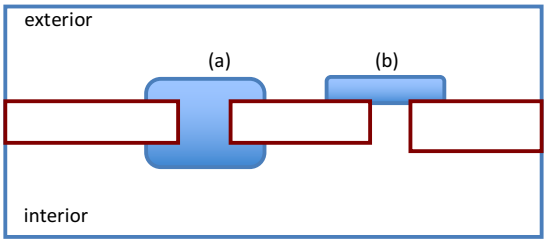


Figure 4.1. Schematic illustrating (a) mode of the new hydrogel plug placement through the scleral tissue, compared to the original vision (b) of a patch placed over the eye tissue. The result is a stronger adhesion.

Milestone 4 Specific Objectives

- Synthesize different polymer chemistries based on the co-polymer compositions
- Inject hydrogel chemistries into the artificial eye for in vitro IOP testing and perform max IOP test.
- Collect and tabulate results to identify best performers

Milestone 4 Significant Results & Key Outcomes

Key Outcome 1: The co-polymer hydrogel compositions were significantly more successful at sustaining IOP in the in vitro model of scleral trauma vs. the pure PNIPAM. *Table 4.1* summarizes the results, and attention should be focused on the second rightmost column which shows the maximum IOP held by each chemistry (Note: all tests were conducted in triplicate and the values are averaged over those three tests).

The pure PNIPAM compositions were unable to prevent saline leakage from the 3mm incisions created on the scleral tissue surfaces in the in vitro model. This was in stark contrast to the copolymer chemistries which were able to hold IOP up to 77mm Hg (Note: 77mm Hg is not the upper limit of the pressure sensor use).

Table 4.1. IOP Adhesion Performance Data for Different Hydrogel Chemistries

Date	Compound	Co-P Ratio	LCST	MW (Avg)	% [Aqueous]	Maximum Pressure Held (mmHg)	Pass/Fail
7.15.2013	PNIPAM	N/A	32	2.864×105 (±2.474%)	0.8	0	F
7.15.2013	PNIPAM	N/A	32	2.864×105 (±2.474%)	2	0	F
7.15.2013	PNIPAM	N/A	32	2.864×105 (±2.474%)	5.26	0	F
9.25.2013	PNIPAM	N/A	32	10,000	10.0	0	F
9.25.2013	PNIPAM	N/A	32	10,000	14.2	0	F
9.25.2013	PNIPAM	N/A	32	10,000	25.0	0	F
9.25.2013	PNIPAM	N/A	32	10,000	30.0	0	F
9.25.2013	PNIPAM	N/A	32	10,000	43.2	0	F
11.22.2013	PNIPAM:n-tert	85:15	25	1.038×10^{6} (±2.583%)	No	No	No
12.6.2013	PNIPAM:n-tert	85:15	25	6.624×10^{5} (±1.506%)	10.0	0	F
12.6.2013	PNIPAM:n-tert	85:15	25	6.624×10^{5} (±1.506%)	15.0	0	F
12.6.2013	PNIPAM:n-tert	85:15	25	6.624×10^{5} (±1.506%)	20.0	40	F
12.6.2013	PNIPAM:n-tert	85:15	25	6.624×10^{5} (±1.506%)	30.0	77	P
10.31.2013	PNIPAM:n-tert	85:15	25	5.55×10^{5} (±1.472%)	10.0	0-10	F
10.31.2013	PNIPAM:butylacrylate	95:5	25	3×10^{4}	10.0	0	F
10.31.2013	PNIPAM:butylacrylate	95:5	25	3×10^{4}	15.0	77.4	P
10.31.2013	PNIPAM:butylacrylate	95:5	25	3×10^{4}	20.0	77.2	P
10.31.2013	PNIPAM:butylacrylate	95:5	25	3×10^{4}	30.0	77.9	P
11.13.2013	PNIPAM:butylacrylate	88:12	14-16	3×10^{4}	10.0	0	F
11.13.2013	PNIPAM:butylacrylate	88:12	14-16	3×10^{4}	15.0	40	F
11.13.2013	PNIPAM:butylacrylate	88:12	14-16	3×10^{4}	20.0	77.2	P
11.13.2013	PNIPAM:butylacrylate	88:12	14-16	3×10^{4}	30.0	N/A (too viscous)	F

Milestone 4 Other Achievements

None to report.

MILESTONE 5: Time to Attach/Detach Test

Milestone 5 Major Activities
The objective of this milestone was to collect performance data on the time to attach/release for the hydrogels.

Milestone 5 Specific Objectives
- Synthesize hydrogel test samples
- In a controlled model/test protocol perform an evaluation of "time to attach" and "time to detach"
- Compile results

Milestone 5 Significant Results & Key Outcomes
Due to the challenges experienced with the polymer chemistry development, and the additional effort needed to identify effective hydrogel chemistries to meet adhesion requirements a controlled and systematic series of time to attach/detach tests were not performed. However, qualitative observations were collected over the course of the project with respect to adhesion and release of the hydrogel.

Key Outcome 1: Based on these observations, all in vivo attachment studies used a 5-minute curing protocol to allow the hydrogel to set in place. That is, from the time of placement of the hydrogel, a five minute "set period" was allotted to allow the hydrogel to setup, before any further work was conducted on the eye. The success of the in vivo study provide validation that this five-minute period is a reliable estimate of the time to attach.

Key Outcome 2: Qualitative observations on the time to detach were performed on selected in vivo test animals at the study endpoint. Two key observations were made: 1) introduction of iced water at the implantation site via water pick/irrigation tool enabled quick release of the hydrogel, 2) the bulk of hydrogel after 48hrs of implantation remained someone rigid suggesting that the hydration from the cold water was limited to the surface of the hydrogel patch and not the bulk of its volume. *Figure 5.1* shows a photograph of a remove hydrogel patch/plug after 48hrs placement. The hydrogel was transparent and rigid. Note that it is emphasized that there was no adhesive resistance to removal after introduction of cold irrigation despite the rigidity.

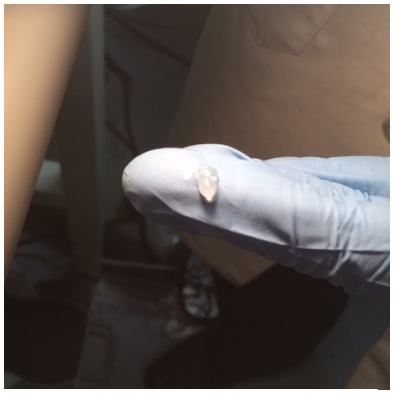


Figure 5.1. Photographic image showing hydrogel plug post-explantation from 48hr placement.

Milestone 5 Other Achievements.
None to Report.

MILESTONE 6: Fabricate Test Patches for In Vivo Testing

Milestone 6 Major Activities
The PNIPAM:butylacrylate copolymer hydrogel was selected as the hydrogel for in vivo testing. Several series of hydrogel were prepared under sterile conditions and packaged for in vivo testing. In addition to this, a separate effort had to be initiated to develop an injector tool to actually deploy the the hydrogel in the animal eyes. The Specific Objectives for this Milestone were modified to include goals for developing a prototype injector tool.

Milestone 6 Specific Objectives
- Develop a polymer chemistry preparation approach under sterile conditions.
- Prepare copolymer hydrogel for in vivo testing
- Design, fabricate and benchtop verify an injector tool for in vivo deployment of the unsupported hydrogels

Milestone 6 Significant Results & Key Outcomes
Key Outcome 1: An approach for sterile hydrogel preparation was developed, see *Figure 6.1*. First, the copolymers were added (in powder form) to a crimp top vial, and the vial along with crimp cap were placed inside a sealed sterilization polybag. The bag was ETO sterilized with the top off the vial to allow penetration of the sterilization gas into the vial. Following sterilization and while still inside the sealed bag, the crimp cap was carefully placed over the vial and crimped while in the bag. Once the vial sealing was confirmed the bag was opened and the vial was labeled. Next a sterile syringe was loaded with sterile DI water (purchased) and the correct volume of sterile water was injected into the vial through the rubber crimp top. The vial was then sonicated to accelerate hydration of the polymer. Once hydrated the sample was transferred to a refrigerator for storage.

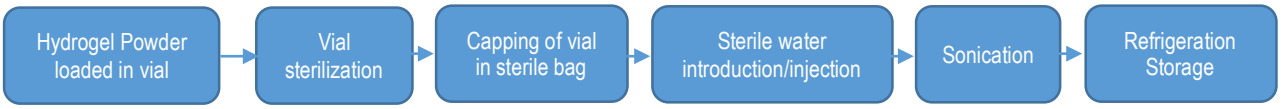


Figure 6.1. Flow chart showing stepwise process of preparing sterile hydrated hydrogel for in vivo validation studies.

Key Outcome 2: Vials of hydrogel were prepared containing approximately 2mL per vial. It was estimated that a single implantation placement would require no more than 0.5mL, and therefore we prepared 2mL in each vial to allow enough material in the even that a second attempt (or more) was necessary for a single animal procedure. Six vials were prepared in the first series.

Key Outcome 3: A preliminary injector tool design was drafted and a prototype, first-generation tool was fabricated. The design was developed by a summer undergraduate research assistant. The design was based on a hydrogel carrier comprised of a 1cc sterile syringe. The syringe was then placed inside an autoclave-sterilized customized 20mL syringe. The volume created between the 20mL syringe and the 1cc syringe was subsequently filled with a mixture of ammonium nitrate and water to induce an endothermic chemical reaction to cool the hydrogel during deployment, *Figure 6.2*. The endothermic reactants were given two minutes to react and bring the hydrogel to the desired temperature. Once ready, a modified, sterile intravenous, polymeric catheter tip was placed onto the end of the 1cc syringe and the hydrogel was deployed on the eye.

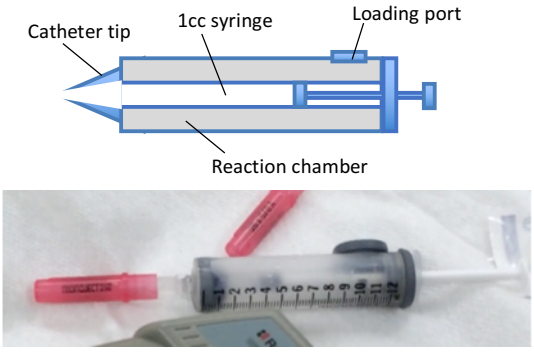


Fig 6.2. Schematic (top) and photograph (bottom) of engineered hydrogel injector tool with integrated, disposable temperature control system.

For each procedure, approximately 0.3cc to 0.4cc of sterile hydrogel was extracted from a sterile crimp top vial using the 1cc syringe in the tool (no needle) with care not to aspirate bubbles into the chamber. Excess hydrogel was wiped way from the tip of the syringe using sterile gauze.

Key Outcome 4. A series of endothermic reaction studies were performed to identify the right mixture of water and ammonium nitrate to enable the tool to cool the hydrogel to <5C for at least 10 minutes. *Figure 6.3* is a representative temperature-time transient recording we collected from the various performance tests we conducted. Thermocouple sensors placed inside the 1cc syringe were used to track temperature vs. time. The red band represents the desired temperature zone for deploying the hydrogel.

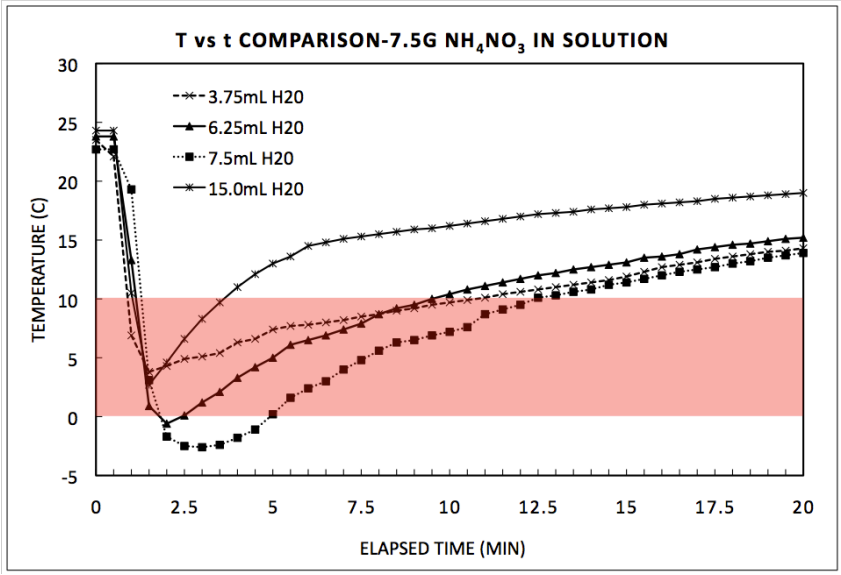


Figure 6.3. Temperature v Time curves recorded for hydrogel chamber in the deployment tool after mixing the endothermic reaction components.

Milestone 6 Other Achievements

We have acquired preliminary verification data on a novel injector tool to support the new hydrogel design.

MILESTONE 7: Identify Best Test Patches

Milestone 7 Major Activities

A preliminary series of in vivo placement experiments were performed to validate the hydrogel chemistry and tool design. Under anesthesia (intramuscular ketamine/xylazine) and topical analgesia (topicaine drops), a small incision was created at the conjunctival junction with the limbus in the temporal quadrant of the right eye (OD). A pocket was created, exposing the scleral surface. A 3mm linear incision (regular margins) through the scleral wall was then created approximately 2-3 mm away from the edge of the limbus and oriented in a direction tangent to the perimeter of the limbus. Topical antibiotic ointment was applied to the OD of the control group subjects and then allowed to recover. Treatment eyes were treated with hydrogel using the deployment tool.

The catheter tip of the injector tool was inserted into the 3mm incision such that the tip was inside the posterior chamber. Pressure was applied to the plunger of the syringe while the catheter tip was slowly withdrawn, creating a spherical node of hydrogel immediately adjacent and interior to the incision, with a trail of hydrogel filling through the incision tract. Once the catheter tip was completely withdrawn, additional hydrogel was deployed onto the exterior surface of the sclera, forming a "rivet-like" structure with hydrogel caps on both interior and exterior surfaces of the sclera. A total of no more than 0.3cc of hydrogel was used for all eyes. An incandescent lamp was positioned near the eye so that the hydrogel surface temperature was held at 32.5C for five minutes. After five minutes, excess hydrogel was trimmed away from the sclera to create a low profile surface. The conjunctiva was then drawn back over the incision area with no sutures placed. *Figure 7.1* shows the procedure in a series of time-lapse photographs.

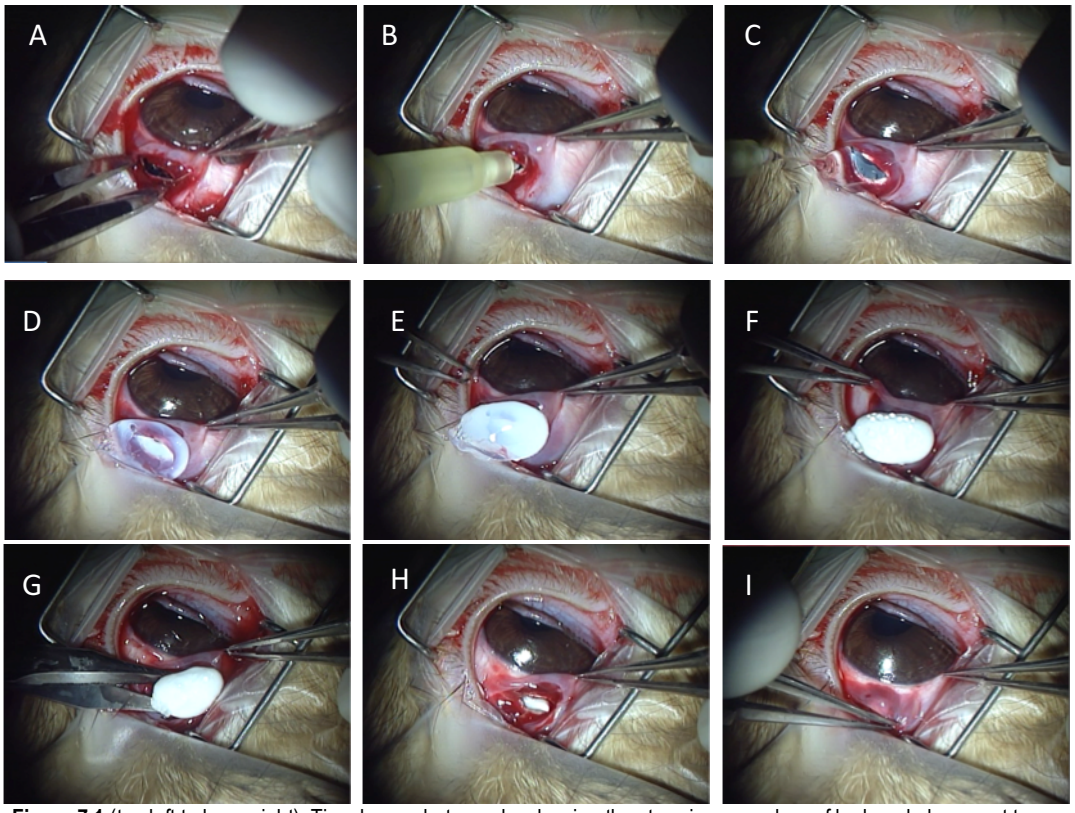


Figure 7.1 (top left to lower right): Time lapse photographs showing the stepwise procedure of hydrogel placement to seal a 3mm non-stellating scleral penetrating injury.

IOP was tracked in the rabbit's eyes for 48-hours after the procedure then it was euthanized and tissue isolated (enucleation) and prepped for histology.

Milestone 7 Specific Objectives

- Prepare sterile hydrogel and deployment tools
- Perform preliminary in vivo placement of hydrogel
- Evaluate animal post-procedure for acute signs of distress/problems.
- Use results to determine how to proceed to expanded in vivo study

Milestone 7 Significant Results & Key Outcomes

Key Outcome 1: Preliminary procedures suggest that the hydrogel-tool-procedure combination that we have developed are able to occlude a 3mm full-thickness laceration to the sclera. In the current design, the tool has to be manually loaded with the endothermic reaction reagents (ammonium nitrate and water) *Figure 7.2(top)*, however, we anticipate this could easily be pre-loaded in separate reservoirs (e.g. a chemical fluorescent lightstick or chemical ice bag which both separate the reactants in breakage chambers which are mixed after breaking.

Clinical user feedback confirmed that we effectively used stakeholder inputs to develop a design that was both usable and met other design requirements. Examples included:

- Ease-of-use with one hand
- Syringe-style design
- Simple cooling strategy
- Hold 0.2 to 0.5mL hydrogel Form factor should be balanced when held in hand
- No electrical components
- Must cool hydrogel in less than 5sec
- Must lower temperature between 0-10C
- Must maintain T < 10C for 10sec+
- Should be held like pencil or syringe
- Weight should be minimal

We have already begun design of a Gen2.0 injector tool, pictured in Figure 7.2 (bottom).

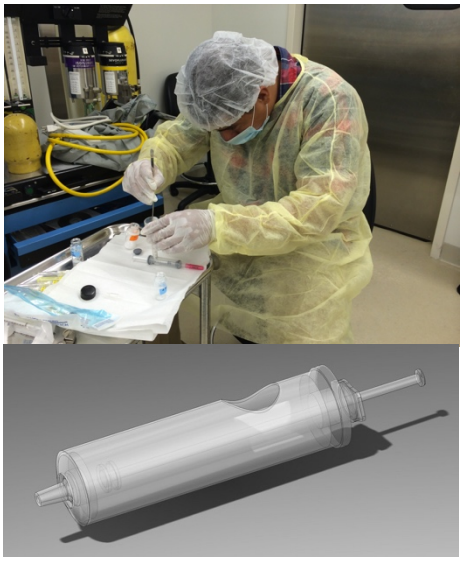


Figure 7.2. Manual loading of the prototype hydrogel injector tool. Future designs would be pre-loaded and ready for use.

Key Outcome 2: An unexpected outcome of this first in vivo procedure was an observation that the hydrogel converts from an opaque white coloration immediately after setting to a transparent appearance after 48hrs placement, *Figure 7.3*. This was an unanticipated outcome from the study and may lend some unique advantages since it may afford medical staff a second visual access point into the posterior segment of the eye (besides the lens).

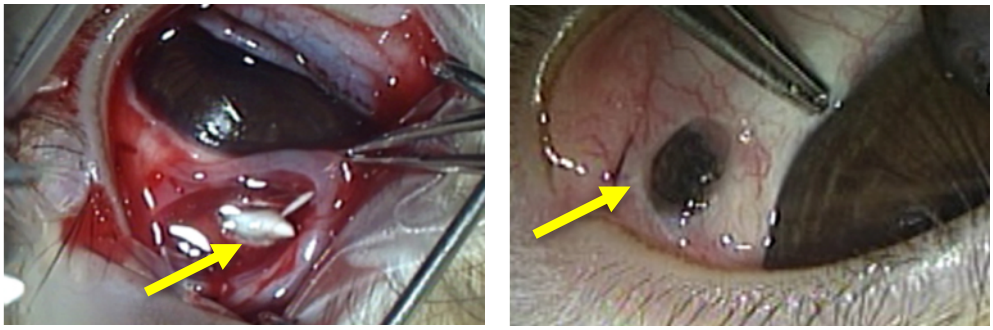


Figure 7.3 The hydrogel (yellow arrows) is opaque and white (top) at time of placement; and (bottom) hydrogel after 48hr placement appears smooth and transparent.

Milestone 7 Other Achievements

None to Report.

MILESTONE 8: In Vivo Sclerotomy Closure Test

Milestone 8 Major Activities

18 rabbits (New Zealand pigmented, 2-4kg) were randomized (2:1) to either treatment group or control. IOP was measured once a day for four days in both right (OD) and left (OS) eyes prior to surgical procedure to establish baseline IOP. All surgical procedures were performed in the AM and completed before noon on Day 0 (implant day). A 3mm linear sclerotomy incision was created in OD of all rabbits (treatment and control), temporal and superior to the cornea approximately 3mm radially away from and tangential to the limbus.

Treatment group rabbits received a 0.2–0.3 mL injection of hydrogel using the Gen 1.0 tool. The stepwise placement was illustrated in *Figure 7.1.* To recap in stepwise detail, first the incision is made and size (3mm) confirmed with

calipers (A). Then the hydrogel is injected into the orifice (B-C) and left for 5 minutes to set (D-F). Temperature rise causes the hydrogel to expel water from its matrix, changing its transparency to opaque white. After 5 minutes, excess hydrogel is trimmed with shears (G) leaving a small external cap (H), and the conjunctiva was drawn over the occlusion (I). After the procedure, rabbits were left to recuperate. The first IOP measurement post-procedure was taken approximately 6 hours later. IOP was measured twice daily (morning, late afternoon) for 3 days following the procedure.

All animals completed the study period with no significant adverse events, e.g. incidences of infection or irritation.

There was no significant difference between the baseline IOPs of the control vs. treated groups ($p = 0.35$).

Milestone 8 Specific Objectives

- Perform simulated scleral trauma surgery on rabbits and deploy hydrogel to seal the penetrations in the treatment group (control group receive no intervention).
- Track intraocular pressure as a function of implantation duration and report as a function of IOP in the lacerated eye vs. IOP in the control eye (not lacerated).
- Report mean IOP vs. implantation duration. Primary Endpoint: Scleral penetrations treated with hydrogel result in a statistically significant improvement in IOP, an indicator of eye stability.

Milestone 8 Significant Results & Key Outcomes

Key Outcome 1: With multiple repeated tests, the hydrogel was easily and repeatable deployed using the prototype tool. After placing 12 rabbits with the hydrogel, user feedback from the surgeon in the vivarium was that the device performed consistently and was able to be use easily to occlude the penetrations. Size, balance, ease of hydrogel release were all attributed that were described qualitatively by the user. The only challenge observed was the slight delay required to prepare each injector tool prior to each placement (discussed previously). We see this as a temporary challenge which should be easily mitigated with future designs.

Key Outcome 2: In an animal model of open globe injury, animals treated with the injectable hydrogel showed an almost immediate increase in IOP to approximately 50% of normal, vs. the control group's IOP which was only 30% of normal ($p<0.05$). The Treatment group IOP improved to almost 60% of normal within 24hrs.

Key Outcome 3: In an animal model of open globe injury, the injectable hydrogel plug demonstrated a statistically significant improvement in mean ocular IOP after treatment of 3mm non-stellating scleral perforations with the hydrogel vs. a cohort which received no treatment. The hydrogel plug caused a statistically significant improvement in mean IOP up to 3 days after surgery, when compared to the control group (0.50±0.05 vs. 0.25±0.04, $p<0.001$).

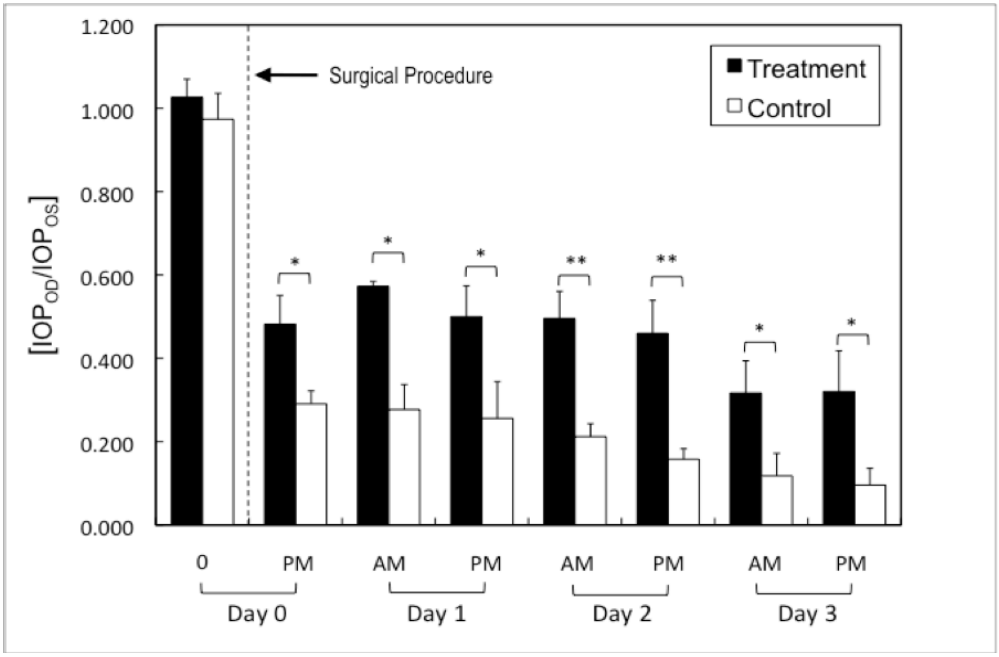


Figure 8.2. Comparison of mean IOP of Treatment vs. Control eyes (OD), normalized by IOP of contralateral eyes (OS) [IOP_{OD} / IOP_{OS}]. Mean IOP was measured for four days prior to surgical procedure (data not shown), with no significant differences between treatment and control ($p = 0.35$). Following intervention, the Treatment group showed a statistically significant higher mean IOP ($P<0.0001$ over the entire course of post-surgical follow-up). Graph shows group mean (SEM) IOP_{OD}/IOP_{OS}; p-values: *$p < 0.05$, ** $p < 0.001$.

Key Outcome 4: No visible or observable signs of discomfort, pain or distress were observed in the treatment group animals after the placement procedure. The afternoon following all morning procedures, all animal exhibited some tenderness and pain and evidenced by a slightly squinted and tearing eye. All rabbits were mobile albeit less active in their cage the afternoon after the procedure. However, the following day all rabbits were mobile and showed no more visible signs of discomfort. No visible matting of fur circumferentially at the eye lids was apparent (suggesting of chronic tearing), and no significant signs of inflammation or irritation were observed during the eye examination, *Figure 8.1*. All rabbits, including those in the control group allowed the research team to perform multiple rebound tonometer measurements on the cornea (3 per eye) without incident. These observations suggest that all animals were ok following the procedure, despite the major drop in IOP for the control group and despite the hydrogel foreign body in the treatment group.

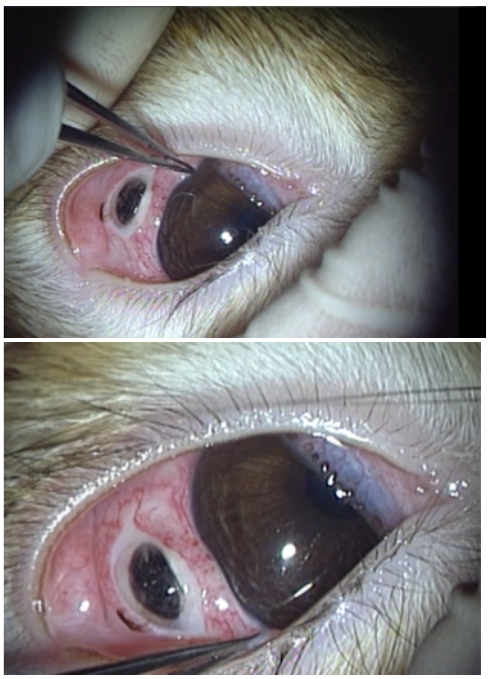


Figure 8.1 Optical micrographs of scleral surface showing hydrogel in situ after 48hrs.

Milestone 8 Other Achievements

An interim analysis of the result of the in vivo study revealed that the difference in IOP between the treatment and control groups was large enough to end the study early, having already met its success criteria. Thus the study was discontinued after 18 animals.

MILESTONE 9: In Vivo Peritomy Test

Milestone 9 Major Activities

We investigated the use of a supported patch chemistry to prevent fibrosis between the scleral tissue and the conjunctiva/Tenon´s capsule. Control rabbits were implanted (OD) with a 2 x 3 mm polypropylene patch subconjunctivally and located approximately 3-5 mm radially away from the limbus in the superior hemisphere. Treatment group animals were implanted (OD) with a 2 x 3 mm pNIPAM-Polypropylene-pNIPAM patch in a similar location. The objective was to determine whether the histological response of the treatment group was equal or better than the response of the control group. **Figure 9.1** shows histological cross sections of the implantation area of the control eye (left) and the treatment eye (right).

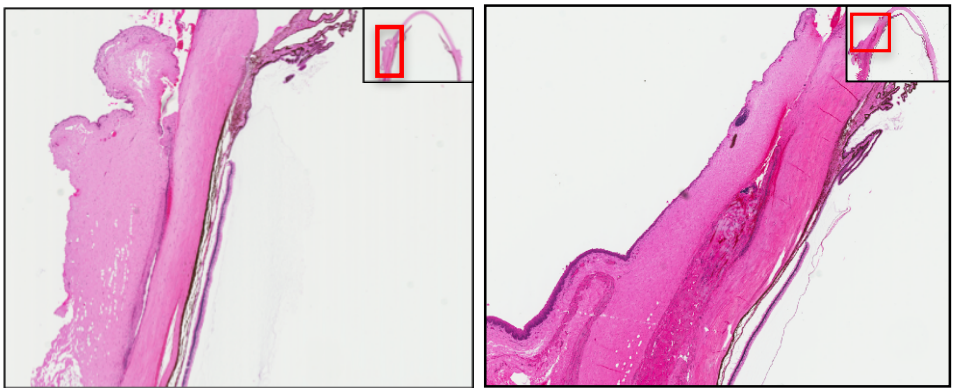


Figure 9.1 Histological cross-sections of the eye wall and conjunctiva following 2-week implantation of a control PP patch (top) and a treatment PNIPAM patch (bottom). Blue arrows denote sub conjunctival space where patches were placed.

Milestone 9 Specific Objectives

- Perform two separate peritomy procedures in the right eyes (OD) of rabbits to create independent subconjunctival pockets, one in the superior hemisphere and the other in the inferior hemisphere.
- Each subconjunctival pocket receives only one of the following possible interventions: 1) a PNIPAM coated parylene substrate patch, 2) a polypropylene control patch, 3) a parylene control patch or 4) no foreign body (treatment control)
- Histological analysis post implantation was performed to assess tissue response in the area.

Milestone 9 Significant Results & Key Outcomes
Key Outcome 1: Our preliminary study showed release of the PNIPAM hydrogel from the parylene substrates and uptake by surrounding cells. The appearance was markedly different compared to the polypropylene substrates. The polypropylene substrates induced only a very localized tissue response, with virtually no indications of material release from the surface nor major accumulations of infiltrate or other signatures of chronic inflammation. We will need to take more time to investigate ways to control/limit the amount of PNIPAM release from the substrate and into the surrounding sub-conjunctival tissue.

Key Outcome 2: Despite the release of PNIPAM from the surface, there were minimal signs of chronic inflammation, even after 30 days of implantation. Only limited numbers of inflammatory markers were visible, and they were not regularly distributed over the entire region of the implantation. Also there is no signs of cytotoxicity or necrosis as a result of the PNIPAM uptake.

Before any additional work is performed in this space effort must be invested in developing ways to ensure that the PNIPAN remains fixed to the substrate surface or resists uptake by the surrounding tissues.

Milestone 9 Other Achievements
None to report.

MILESTONE 10: Histology

Milestone 10 Major Activities
Enucleated eyes from rabbits participating in the IOP study (Milestone 8) were fixed and prepared for histological examination to assess tissue response to the hydrogel material. The eyes were fixed in 10% formalin solution and subsequently sectioned to acquire cross-sectional views of the sclera at the hydrogel placement. Tissues slides were stained using either hemotoxylin & eosin staining solution or Masson's trichrome stain to assess degree and distribution of inflammatory cells and to track collagen and encapsulation layer thickness. Histological time points evaluated were: 48hr, 1 week and 4 weeks.

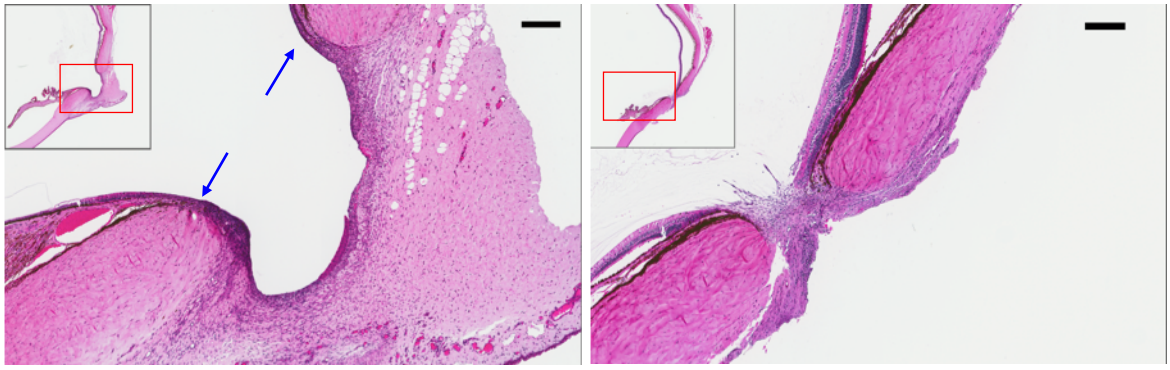


Figure 10.1 (left) Histological cross-section of scleral fistula (edges marked by blue arrows) created by hydrogel placement after 7-days implantation, with low magnification (inset) provided for orientation. (right) 7-Day histological cross-section of scleral fistula that was allowed to heal without intervention (control), showing tissue in-growth closing fistula. Scale bars (top right of each image) are 200um.

Milestone 10 Specific Objectives
- Fix enucleated eyes
- Select tissue staining protocols and send series for staining.
- Review stained slides qualitatively for inflammatory response at implantation sites.
- Review stained slides for encapsulation thickness evaluation.

Milestone 10 Significant Results & Key Outcomes
Key Outcome 1: At 48 hours, the scleral tissue shows a clear interruption in both groups. The inner retinal tissue (visibly layered thin layer indicated by arrows) can be seen beginning to migrate through the laceration outwards. No significant signs of accumulated infiltrate are visible and this may be due to the thin nature of the sclera. In the control eyes, the internal contents (vitreous) may be able to wash the area of any accumulated infiltrate because it is continuously exuding from the laceration.

Key Outcome 2: At one week, markers of acute inflammation are present, and a clear differentiation in the two eyes is apparent. In the treatment eyes, the sclera has created a compact and immature encapsulation layer which

separates the scleral tissue from the implant. The layer is thicker, anywhere from 10 to 20 cell layers in thickness. It also appears that in addition to some migration of retinal layer cells from the inside, it appears that some external epithelial cells may be migrating inward along the tract. The encapsulation layer is immature and contains a number of inflammatory cells through its thickness [8,9].

Key Outcome 3: In comparison to the treatment group, the control group eyes at one week have begun to form a very thin, leaky fibrotic patch through the orifice. It is leaky because the <IOP> measurements for these rabbits are still <4mm Hg at one week. It is also noted that these regions are not easily located during exploration. Colored sutures had been thrown into the surrounding tissue by the surgeon to help with relocation, however the surgeon commented that it would have been very easy to overlook/miss these early scars. Cross sectional histology shows these bridges to be less than 50% the average scleral thickness and rich with inflammatory cells.

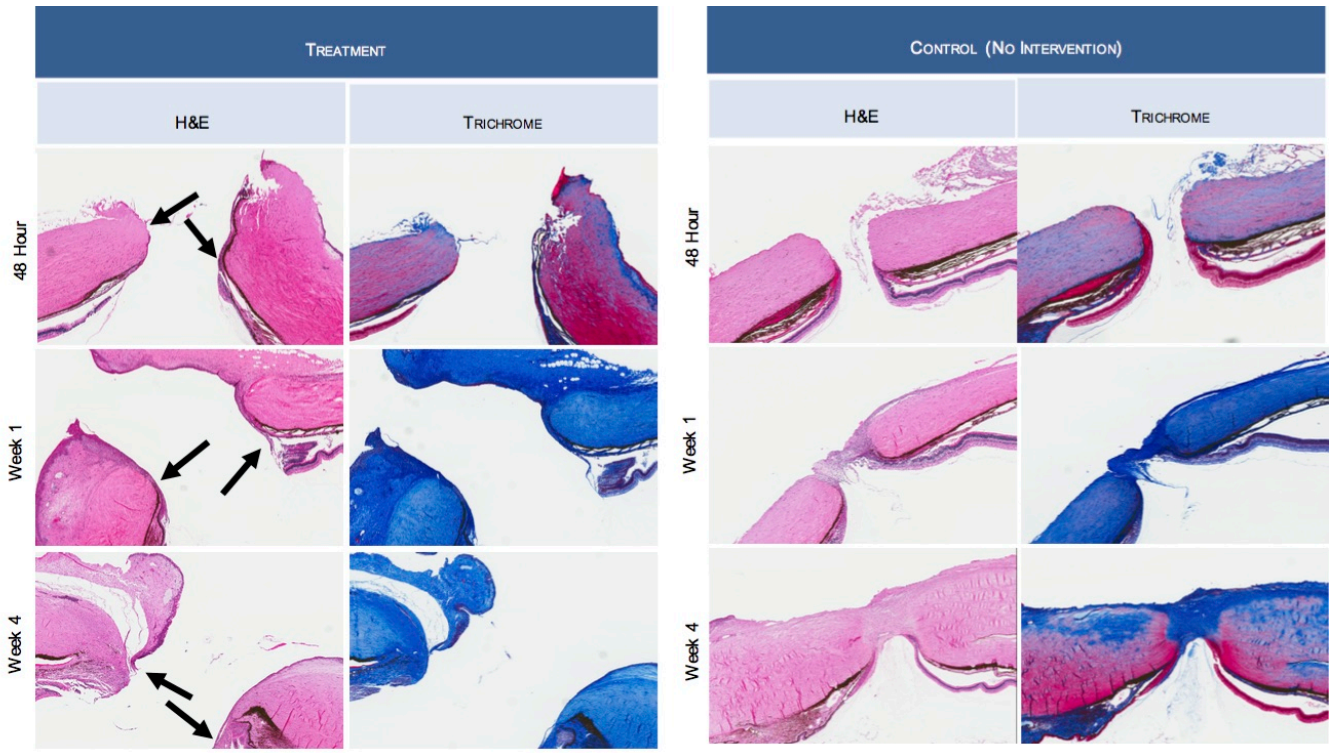


Figure 10.2. (left) Representative histological cross-sections of scleral tissue at the laceration/implantation site for the treatment and control groups. Cross-sections are from rabbits followed for 48hrs, 1-week and 4-weeks, showing tissue cross-sections in both H&E and trichrome stains.

Key Outcome 4: At 4 weeks the scleral wall have developed a mature encapsulation layer of only 1-2 cell layers in thickness suggesting a healthy transition from acute inflammation to a stable foreign body response. There is no indication of necrosis around the site or widespread accumulation of chronic inflammatory markers. Please Note, while the intended use for this technology is on the 1-7 day range, we conducted this 30-day evaluation to assess the complete foreign body response of the eye to the novel copolymer composition. These results are very encouraging and give us hope that further cytotoxicity testing and biocompatibility testing will yield similar positive results [8,9].

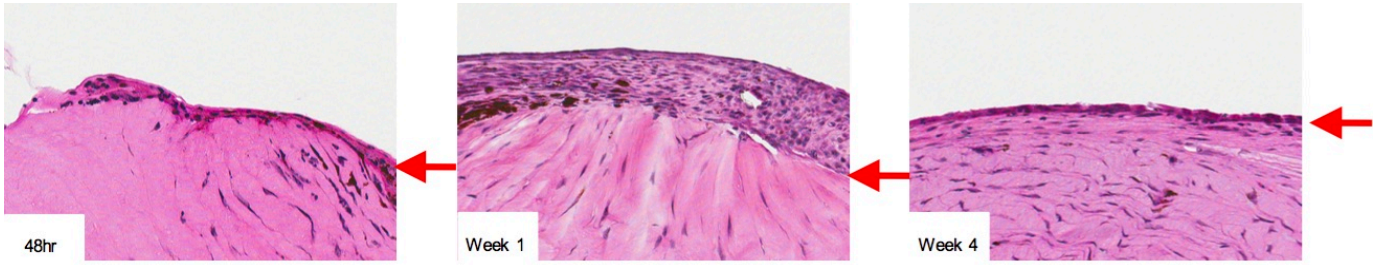


Figure 10.3. Higher resolution micrographs of H&E stained scleral cross-sections for the treatment group at 48hr, 1-week and 4-weeks. Encapsulation layer maturation can be tracked over the 4-week period from preliminary fluid and cellular infiltration to acute inflammatory response forming a thick encapsulation layer, followed by maturation and formation of a compact and mature encapsulation layer devoid of signs of chronic inflammation.

Key Outcome 5: In comparison the the treatment group at 4 weeks, the control histology shows a mature fibrotic scar has occupied the space of the laceration. The trichrome stain shows that the bridging tissue is rich in collagen (evidenced by the heavily blue stained tissues). Also, it can be seen that despite the restoration of the rabbit's IOP to near normal levels at 4 weeks, the cross-sectional thickness of the fibrosis is only 50% that of the scleral wall and the tissue structure is significantly different from the surrounding scleral tissue. While no mechanical studies have been performed, it is likely that there are large variations in the mechanical properties of the scleral tissue in this region.

The results of the tissue response to the hydrogel can be seen more closely in the second series of photographs in *Figure 10.3*.

Milestone 10 Other Achievements
None to report.

MILESTONE 11: Review and Summary Report

This final report is the summary report for this project. No additional notes provided.

c. Opportunities for Training and Professional Development Provided by this Project.
In July 2014, graduate student Niki Bayat from Mark Thompson's group attended the 2014 2nd Annual Workshop on Micro- and Nanotechnologies for Medicine: Emerging Frontiers and Applications in Boston MA and hosted by Harvard University, Brigham and Women's Hospital, Northeastern University and the MIT International Science and Technology Initiatives. The topics covered in this workshop include: biomaterials, microfluidics, drug delivery, tissue engineering and professional development/translation to products.

One area that Ms. Bayat specifically went to learn about was

Our research group has participated in three different summer research programs which provide research experience for high school students (SHARP), research experience for undergraduate teachers (RET), and for undergraduates who are military service veterans (REV).

2014 and 2015 Research Experience for Undergraduate Teachers (RET).
Over the summers of 2014 and 2015, Dr. Humayun's research group participated in the USC-supported and NSF-sponsored "Research Experience for Teachers" (RET) program. The RET program pairs faculty from local community colleges and high schools with research teams on campus to provide the teachers with applied research experience on which they can develop curricula for their students.

In 2014 and 2015, the Humayun Group was paired with Mr. Kamyar Khashayar MS, assistant professor of engineering and Director of the ELAC Engineering Transfer Pathway Grant. During his time with Mr. Khashayar provided key supported in design, fabrication and testing of the injector tool developed to deploy the hydrogel adhesives during testing. Mr. Khashayar's input as a reviewer was critical in creating these original prototypes. He now uses his experience on this project to develop new teaching modules for his class on Computer-Aided Design (CAD) and 3-D printing. After his experience in 2014, Mr. Khashayar requested to be teamed with the same group again in 2015.

Figure 11.1 ELAC professor and USC summer research assistant, Mr. Kamyar Khashayar presenting his final report to the summer research team at the conclusion of the 2014 USC RET program.

2014 Research Experience for Veterans Program.

In 2014, Dr. Joseph Cocozza and Dr. Jack Whalen submitted a proposal to NSF for supplementary funding to support a research experience for undergraduate military veterans, and received an award. Through this program, Mr. Jose Cortez, a California State University - Long Beach undergraduate was paired with the TATRC research team in Dr. Humayun's research laboratory. Mr. Cortez, a former machinist with significant experience in Computer-Aided Design (CAD), was paired with Mr. Khashayar and given the task of designing and producing a series of different injector tool prototypes. Over the time period, Mr. Cortez was able to quickly read a variety of background materials on combat casualty care, thermoresponsive polymers and biomedical engineering, and was able to develop a working prototype which was used in all of our in vivo testing studies, (see Figure).

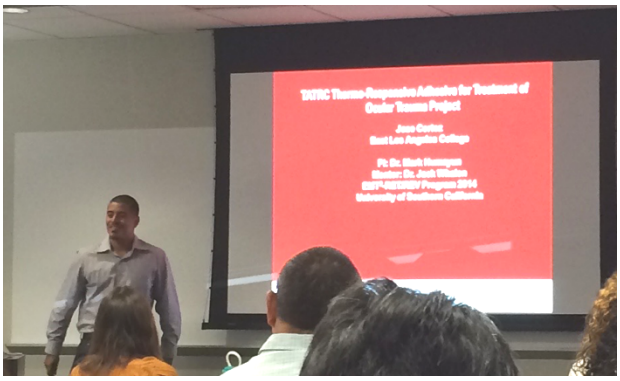


Figure 11.2 US Navy veteran, former ELAC student and current CSULA undergraduate Mr. Jose Cortez presenting his final research report to the USC summer research team at conclusion of the program.

Over the course of the program, Mr. Cortez has helped with managing the research team machine shop, while also receiving training on in vivo animal research, and biomedical engineering research. Mr. Cortez is currently completing his 3rd year at CSULA, and continues to work as a research assistant in the Humayun group.

2015 Summer High school Advanced Research Program (SHSARP).

Over the Summer of 2014 the Humayun group participated in a private trust supported summer research experience program for high school students led by Dr. Joseph Cocozza and titled, "Summer High School Advanced Research Program" or SHSARP. The program, supported by USC's Keck School of Medicine and Viterbi School of Engineering is an 8-week immersive program where high school students are paired with university research teams to provide them with working experience in academic research.

Ms. Jahlyn Reyes-McKinley, a rising senior from King/Drew Magnet High School of Medicine and Science (Los Angeles, CA) was paired with Dr. Humayun's group and helped with our TATRC vision research program. For 8-weeks, Ms. Reyes-McKinley worked closely with our TATRC research team, learning about intraocular pressure, ocular trauma, combat medicine, hydrogels, and in vivo experiments. She took actual IOP measurements on rabbits using a rebound tonometer and generated database spreadsheets to track IOP. Ms. Reyes-McKinley graduated high school in Spring 2015 and is now an undergraduate majoring in engineering at Case Western Reserve University.

d. Results Reporting/Dissemination

Key findings from this program were reported at major relevant research meetings as well as at vision research advocacy meetings on Capitol Hill.

03/2014: Congressional Briefing by Dr. Humayun titled, "Development of a Thermo-Responsive Patch for Ocular Trauma" as part of the AEVR-sponsored "Decade of Vision 2010-2020 Initiative".
05/2014: Association for Research in Vision and Ophthalmology (ARVO). Poster presentation by Y. Zhang
05/2015: Association for Research in Vision and Ophthalmology (ARVO). Poster presentations by Y. Zhang and J Whalen.
08/2015: Military Health System Research Symposium (MHSRS). Poster presentation by J. Whalen
10/2015: Alliance for Eye and Vision Research Emerging Vision Scientists Event on Capitol Hill (AEVR). Poster presentation by J. Whalen

e. Plans for Next Reporting Period

This is a Final Report. Nothing more to report.

4. IMPACT

SUMMARY: This program has contributed to basic knowledge in the fields of polymer chemistry, combat casualty medicine and biomaterials. This basic knowledge may be applicable to other areas of clinical medicine and biomedical engineering like drug delivery and tissue engineering. Since we recognize the potential for commercialization, an effort to capture intellectual property and to engage commercialization partners has been initiated.

a. Impact on Principle Disciplines

The three primary disciplines covered by the research performed in this study are: polymer synthesis research, ocular trauma intervention research, and biomaterials research. The following significant advances and contributions were made to the base of knowledge and understanding in each discipline.

Thermo-responsive Polymer Chemistry Research.

We have expanded the knowledge and understanding of the physical properties of two specific series of co-polymers based on poly N-isopropyl acrylamide (PNIPAM). We have characterized temperature dependence of viscosity and adhesion to scleral tissues. And we have performed the first biocompatibility studies on these materials. This collection of work may serve as a basis for a novel series of implantable materials for biomedical applications, e.g. cell culture, drug deliver, tissue scaffolding, etc. Poly N-isopropyl acrylamide is a well-studied thermo-responsive hydrgogel, with over two decades of publications, however the chemistries we are exploring have not been studied for biomedical research applications.

Ocular Trauma Intervention.

Results from this study suggest that intervention of open globe injuries to the sclera may be intervened at a much earlier time point by either emergency room physicians or potentially by medics/emergency medical technicians. This departs from the current standard of care for managing ocular trauma in the combat casualty setting, and may potentially improve outcomes.

Currently the standard practice in combat casualty care for ocular trauma involves: 1) performing a preliminary assessment of condition (e.g. assessment of the total level tissue damage to determine if the eye is salvageable; removal of large, visible foreign bodies); initiation of antibiotics; and shielding of the eye. Any major intervention is delayed until the casualty can be seen by an ocular specialist.

Biomaterials Research.

The copolymer chemistries we have synthesized in this program have not been previously evaluated for implantation applications. This is the first known study that has collected and reported the results of tissue reaction to this new series of hydrogel materials. While the primary focus of this effort is for ocular applications, we hope that this collection of knowledge may be used as a basis to expand to other implantation locations/applications.

b. Impact on Other Disciplines

Thermoresponsive hydrogels have been explored as scaffolds for tissue engineering and as carriers for drug delivery. Dr. Thompson is also collaborating with a different group to explore dermatological applications.

c. Impact on Technology Transfer

The results of this program have been presented to two manufacturers in the medical device/pharmaceutical industries, with the purpose of exploring collaboration and possible commercialization. Both industry partners included letters of support for the next phase of research and development of this project, which was submitted to the Joint Warfighter Medical Research Program.

A patent and patent application have already been filed/issued related to this material technology (US/8,664,463 and US 12/363,594) and a third provisional application was filed in January 2014 and refiled again in February 2015 to capture new intellectual property developed in this project.

d. Impact on Society Beyond Science and Technology

Nothing to Report.

5. CHANGES/PROBLEMS

SUMMARY: Over the course of this program, we encountered two challenges which resulted in some changes to the original program/plan and a delay in its completion: 1) we found that an injectable plugging hydrogel in free form was more effective than the originally proposed adhesive patches; and 2) an administrative transition in the university's Ophthalmology Department required moving of our laboratory and hiring of new veterinary staff which created some delays in our in vivo study work.

a. Changes in Approach and Reasons

The originally proposed patch design did not perform on par with a commonly used bioadhesive (cyanoacrylate), which we thought was a good benchmark for success. In both in vitro IOP test and uniaxial tension tests, the patch

design performed with less than 20% adhesion strength compared to the cyanoacrylate glue. We did not proceed with the patch design to in vivo studies because of this.

It was then demonstrated that adhesion performance could be attained by using a free-form hydrogel to occlude the lacerations. This approach was effective in the in vitro IOP tests we designed and the same results were confirmed in the in vivo testing we performed.

This change in approach required two technical changes: 1) a change in the polymer chemistry, and 2) design and engineering of a deployment tool to carry and deploy the hydrogel adhesive.

A change in the technical description of the proposed ocular trauma intervention was required. This also led to a delay in our ability to transition to in vivo studies according to the original schedule. However, the proposed indications for the technology remained the same, and the enabling technology continued to be a thermo-responsive hydrogel adhesive, albeit a slightly modified chemistry.

b. Actual/Anticipated Problems/Delays and Actions/Plans to Resolve Them

The second challenge we encountered with this program was an unanticipated administrative problem with the separation of the Doheny Eye Institute from University of Southern California's Department of Ophthalmology. The separation led to a required move of our research laboratories to new space and also a los of vivarium staff and supplies access which caused a delay of approximately 3 months in our program at the 7th quarter of the program.

Shortly after, new staff were hired and oriented in the vivarium and our in vivo studies continued without further delays. The result of the unanticipated administrative issue was a 3-month delay in the research program.

c. Changes that had Significant Impact on Expenditures.

None to report. This program has remained on budget.

d. Significant Changes in Use of Care of human subjects, vertebrate animals, biohazards, and/or select agents.

One change that was made to this study was that the number of rabbits used for the IOP study was reduced significantly because the interim analysis performed by our biostatistician indicated that we had reached success criteria after only enrolling 18 rabbits in the study. This allowed us to reduce the total number of animals used in this research study.

It was discovered that the original IACUC-approved protocol was not submitted to ACURO for review and therefore a protocol deviation notification was submitted to ACURO and an investigation initiated in September 2015. ACURO has requested that all of the animal records associated with the execution of this study be submitted to ACURO for review. We are currently in the process of completing this.

6. PRODUCTS

SUMMARY: Conference presentations and policy maker presentations have been made to share our discoveries research with others. Disclosures have been balanced with the need to capture intellectual property.

a. Publications, Conference Papers and Presentations.

Conference Papers

(All conference papers have acknowledged federal support)

J.J. Whalen III, N. Bayat, Y. Zhang, P. Fallabella, J.R. Cortez, M.E. Thompson, M.S. Humayun. "In Vivo Validation of a Thermoreversibly Attachable Hydrogel for Temporary Repair of Perforating Injuries in Ocular Trauma." Military Health System Research Symposium. Ft. Lauderdale, FL; August 2015.

Y. Zhang, P. Falabella, N. Bayat, S. Rauen, J.D. Weiland, J.J. Whalen III, M.E. Thompson and M.S. Humayun. "Thermoresponsive Reversible Adhesive for Temporary Intervention in Ocular Trauma Utilizing a Rabbit Model." *Invest Ophthalmol Vis Sci* 2015; 56(7): 1603.

J.J. Whalen, Y. Zhang, P. Falabella, N. Bayat, M.E. Thompson, M.S. Humayun. "Polymeric Shield to Reduce Conjunctival Scarring and Facilitate Re-Access for Multistage Surgical Procedures" Invest. Ophthalmol. Vis. Sci. 2015; 56(7): 3042.

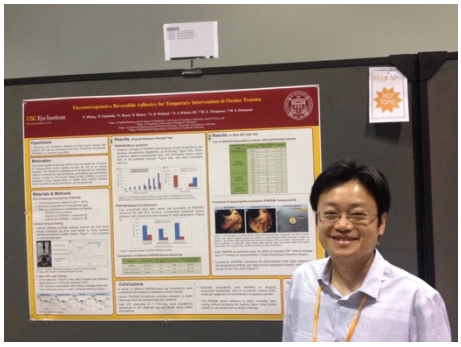
Y. Zhang, J.J. Whalen, P. Falabella, N. Bayat, M.E. Thompson and M.S. Humayun. "Efficacy Studies of Thermoresponsive Reversible Adhesive for Temporary Intervention in Ocular Trauma." *Invest Ophthalmol Vis*

Sci 2014; 55: 2353.

Presentations

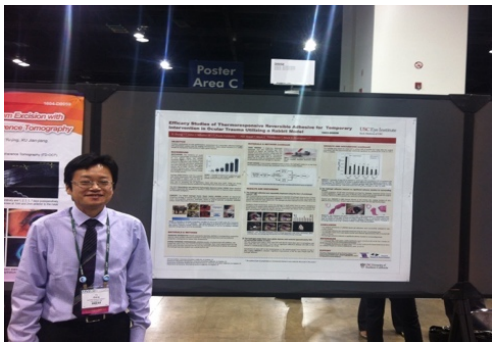
J.J. Whalen. "Novel Biomaterials in Ophthalmology to Treat Ocular Trauma and Infection." 2015 AEVR Emerging Scientist Research Event. Capitol Hill. October 7, 2015.

M.S. Humayun. "Thermo-Responsive Patch for Ocular Trauma." AEVR's Decade of Vision 2010-2020 Initiative. Capitol Hill. March 6, 2014.



Dr. Yi Zhang poster presentation at ARVO 2014.

Dr. Mark Humayun presentation at AEVR Capitol Hill visit in March 2014.



Dr. Yi Zhang poster presentation at ARVO

Dr. Jack Whalen poster presentation at AEVR Capitol Hill visit in October 2015.

b. Websites and other Internet Sites.

http://www.eyeresearch.org/naevr_action/March_2014_VTRP_briefing.html

c. Technologies or Techniques.

New Polymer Hydrogel Chemistries.

A series of new polymer hydrogel chemistries have been synthesized, each with unique physical characteristics, e.g. viscosity, glass transition temperature, etc.

We hope that this library of new co-polymer chemistries and collection of physical properties data may serve as a basis and reference for design of other biomedical technologies and/or devices.

New injector Tool for Hydrogel Deployment.

We have developed a novel injector tool to deploy the designed hydrogels. This tool employs a number of design attributed that were developed based on user needs, as well as performance and engineering constraints that were developed by our team of clinicians and engineers. The details are not provided here as we are building an intellectual property portfolio around the device.

d. Inventions, Patent Applications or Licenses.

Provisional application was filed 31-JAN-2014 with the US Patent Office (Application No. 61934061).

The provisional was refiled on 02-FEB-2015 (Application No. 62110851).

e. Other Products.
None to Report.

7. PARTICIPANTS & OTHER COLLABORATING ORGANIZATIONS

Name:	Mark Humayun, MD PhD
Project Role:	Principal Investigator
Research Identifier:	None.
Nearest Person Month Worked:	1
Contribution to Project:	Dr. Humayun was the principal investigator and oversaw the overall execution of the program. He reviewed all research results and reports.
Funding Support:	2% from TATRC VRPI; Other support from: California Institute for Regenerative Medicine National Science Foundation National Institutes of Health.

Name:	Mark Thompson, PhD
Project Role:	Co-Investigator
Research Identifier:	None.
Nearest Person Month Worked:	1
Contribution to Project:	Dr. Thompson is responsible for overseeing and guiding polymer chemistry synthesis and characterization.
Funding Support:	National Science Foundation Department of Energy Nanoflex Power Corp. (industry sponsor) Universal Display Corp. (industry sponsor)

Name:	John (Jack) Whalen, PhD
Project Role:	Key Personnel/Researcher
Research Identifier:	None.
Nearest Person Month Worked:	20
Contribution to Project:	Dr. Whalen has project managed the program effort including overseeing drafting of animal protocols, review of
Funding Support:	TATRC: 50%; Other support from: National Science Foundation Department of Defense SBIR National Institutes of Health SBIR

Name:	Yi Zhang, PhD
Project Role:	Post-Doctoral Researcher (biomedical engineer, materials scientist)
Research Identifier:	None.
Nearest Person Month Worked:	10
Contribution to Project:	Dr. Zhang has led design setup and execution of uniaxial in vitro testing, IOP in vitro testing and supported in vivo implantation studies.
Funding Support:	TATRC: 25%; Other support from: Department of Defense SBIR National Institutes of Health

Name:	Paulo Falabella MD
Project Role:	Post-Doctoral Researcher (Ophthalmologist/surgeon)
Research Identifier:	None.
Nearest Person Month Worked:	6
Contribution to Project:	Dr. Falabella was responsible for performing all surgical procedures as well as post-surgical evaluations, and enucleations for tissue histology analysis.
Funding Support:	

Name:	Niki Bayat
Project Role:	PhD graduate student research assistant (Chemistry)
Research Identifier:	None.
Nearest Person Month Worked:	36
Contribution to Project:	Ms. Bayat has spearheaded polymer chemistry synthesis, materials characterization of the polymer chemistries and supported sample preparation for all in vitro and in vivo studies.
Funding Support:	100% TATRC

Name:	Stacey Rauen, MS
Project Role:	MS Graduate student research assistant
Research Identifier:	None.
Nearest Person Month Worked:	12
Contribution to Project:	Ms. Rauen was responsible for setting up and collecting uniaxial tension testing data as well as in vitro IOP measurements.
Funding Support:	100% from National Science Foundation

Name:	Kamyar Khashayar MS
Project Role:	Summer Research Assistant
Research Identifier:	None.
Nearest Person Month Worked:	9
Contribution to Project:	Mr. Khashayar is a summer research assistant who helped with design and fabrication of the injector tools for the unsupported hydrogel.
Funding Support:	NSF Research Experience for Teachers supplemental grant (100%).

Name:	Jose Cortez
Project Role:	Undergraduate summer research assistant
Research Identifier:	None.
Nearest Person Month Worked:	9
Contribution to Project:	Mr. Cortez was responsible for designing and fabricating the first prototype injector tools for the unsupported hydrogel.
Funding Support:	NSF Research Experience for Veterans supplemental grant (100%).

Name:	Jahlyn Reyes-McKinley
Project Role:	High school summer research assistant
Research Identifier:	None.
Nearest Person Month Worked:	2
Contribution to Project:	Ms. Reyes-McKinley was a summer high school researcher who supported our team by helping with performing intraocular pressure measurements on rabbits post-implantation, as well as tabulating our IOP data.
Funding Support:	Private Foundation support 100% (Windsong Foundation)

8. SPECIAL REPORTING REQUIREMENTS

Please see attached Quad Chart.

9. APPENDICES

Thermo-Responsive Reversibly Attachable Patch for Temporary Intervention in Ocular Trauma

Problem (Vision Gap One): Early Intervention and Mitigation Strategies to Reduce Further Injury and Slow or Stop Loss of Vision.

Contract No. **W81XWH-12-1-0341**

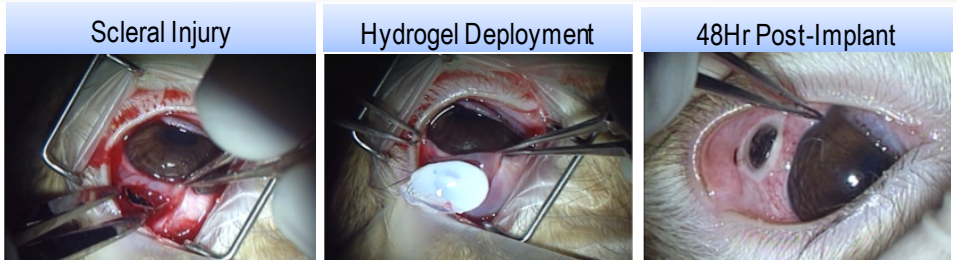
PI: Mark Humayun, MD PhD **Org:** University of Southern California **Award Amount:** $814,121

Study/Product Aim(s)

- Perform performance assessment studies on a novel, biocompatible, reversibly adhesive patch based on PNIPAM hydrogel that meets the most significant safety and efficacy concerns for treatment of combat-related ocular trauma.
- Device key features that would be useful in combat scenario:
  (1) Easily reversible upon cooling without damaging the healing tissue.
  (2) Ease-of-use, allowing sutureless wound closure enabling treatment in lower echelon care centers.

Approach

The objectives of this program were to: 1) synthesize and characterize adhesion and other physical properties of PNIPAM-based hydrogel adhesives, 2) characterize sterilization effects on adhesion, 2) characterize environmental effects (temperature) on adhesion, 3) perform in vitro and in vivo adhesion performance characterization and 4) perform preliminary biocompatibility assessment through histological evaluation vs. implantation time. The goal was to determine whether this technology may be a viable option for addressing time-sensitive open globe injuries to the eye wall.



Accomplishments:

- Unsupported hydrogels of a copolymer chemistry have been developed which are sterilization stable and exhibit desired thermoresponsive characteristics.
- A low-cost disposable injection tool has been designed, fabricated and validated for use in an in vivo model
- An in vivo assessment of the hydrogel patch performance showed a statistically significant improvement over the current standard of care (no intervention)
- Histological evaluation shows the hydrogel induces no major adverse tissue response within 30 days of implantation.

Timeline and Cost

Activities	CY13	CY14	CY15
1) Does pNIPAM thickness impacts adhesion;			
2) Does standard medical sterilization processes adversely impact adhesion;			
3) Does extreme temperature exposure adversely impacts patch performance;			
4) Is patch capable of meeting preliminary ease-of-use design criteria;			
5) Determine if patch can repair scleral trauma in vivo;			
6) Determine if patch can prevent scleral-conjunctival scarring post peritomy;			
Estimated Budget ($)	**$227,478**	**$228,506**	**$358,137**

Updated: 15 Nov 2015

Goals/Milestones

CY12 Q4 Goal: – Fabrication, Quality Characterization, Exposure Test

- ☑ Fabricate pNIPAM-parylene of different thicknesses (h=100nm, 400nm, 800m)
- ☑ Perform baseline characterization (contact angle vs. T and FTIR)
- ☑ Sterilize patches using either ETO sterilization or autoclave sterilization protocol
- ☑ Extreme Thermal Exposure of Patches (120°F or -50°F for 168hrs)
- ☑ Post-exposure characterization (Contact Angle vs. T and FTIR)

CY13 Goal – Performance Evaluation

- ☑ Evaluate effect of sterilization and temperature protocols on adhesion characteristics; Evaluate ease of use of patches
- ☑ Initiate in vivo studies for scleral penetration closure and peritomy scarring prevention.

CY14 Goal – Biocompatibility Assessment

- ☑ Complete in vivo studies and analyze data for summary report

Comments/Challenges/Issues/Concerns

- Delamination of PNIPAM from substrate in peritomy study requires additional work.

Budget Expenditure to date:

Projected Expenditure (Year 2): $814,121 Actual Expenditure: $780,500

11. REFERENCES

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