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Title: Thermoresponsive Reversible Adhesive for Temporary Intervention of Ocular Trauma - II

PRINCIPAL INVESTIGATOR: **Mark S. Humayun MD PhD**

CONTRACTING ORGANIZATION: University of Southern California
Los Angeles, CA 90089

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Mark S. Humayun, Mark E. Thompson, John J Whalen III

E-Mail: humayun@med.usc.edu

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University of Southern California
3720 S. Flower Street. Third Floor
Los Angeles, CA 90089-0001

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14. ABSTRACT

A pre-clinical research and development project to advance a novel technology for temporary treatment of ocular trauma in austere environments. Open globe injuries, e.g. penetrating injuries to the sclera (the white surface of the eye) require microsurgical instrumentation, trained ocular surgery specialists to perform the closure and time to perform the procedure. We have developed a reversibly deployable, thermo-responsive hydrogel adhesive that can be placed, in a liquid gel form, through the penetrating injury to fill the laceration in the wall of the eye. As the hydrogel adhesive's temperature rises with body temperature, it transitions from a fluid gel into a viscous semi-solid that bridges and seals the penetrating injury for up to thirty days. The entire procedure from injection to fixation can take less than five minutes. Once transported to a ROC4 facility, a specialist can easily remove the adhesive by rehydrating with cold water and aspirating the adhesive away and perform a complete revision. This project pursues four objectives: 1) refine and manufacture a 2nd prototype of the hydrogel and accompanying instrument for subsequent GLP validation testing. 2) Perform GLP biocompatibility and in vivo performance testing of these devices to support a subsequent regulatory package. 3) Hold a pre-submission meeting with the FDA to get preliminary feedback on the product designs, proposed indications and clinical safety assessment strategy. 4) Further explore improvements to the adhesive properties of the hydrogel for potential future developments. To date we have made advanced in all four areas in line with our set goals.

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1. INTRODUCTION:

This is a pre-clinical research and development project to further advance a novel technology for temporary treatment of ocular trauma in austere environments. Open globe injuries, e.g. penetrating injuries to the sclera (the white surface of the eye) require microsurgical instrumentation, trained ocular surgery specialists to perform the closure and time to perform the procedure. We have developed a reversibly deployable, thermo-responsive hydrogel adhesive that can be placed, in a liquid gel form, through the penetrating injury to fill the laceration in the wall of the eye. As the hydrogel adhesive's temperature rises with body temperature, it transitions from a fluid gel into a viscous semi-solid that bridges and seals the penetrating injury for up to thirty days. The entire procedure from injection to fixation can take less than five minutes. Once the patient can be seen by the appropriate specialist with the necessary equipment and time, the hydrogel can be easily removed by simply cooling and rehydrating the adhesive with cold water. This project seeks to accomplish four primary goals: 1) refine and manufacture a first prototype of the hydrogel and accompanying injector instrument for subsequent GLP safety and performance testing. 2) Performance of GLP biocompatibility and in vivo performance testing on the manufactured prototypes to support a subsequent regulatory package. 3) Hold a pre-submission meeting with the FDA to get preliminary feedback on the product designs, proposed indications and clinical safety assessment strategy. 4) Further explore improvements to the adhesive properties of the hydrogel for potential future developments.

2. KEYWORDS:

- Thermoresponsive reversible hydrogel
- Open globe injury
- Penetrating injury
- First point of care
- Ocular trauma
- Intraocular pressure
- Preclinical evaluation
- In vivo validation

3. ACCOMPLISHMENTS:

Project Major Goals for 2017:

	Deliverables due by Q4 2017	Status
1. **Collect used needs and product specifications from combat medical community**	• Define new specs and integrate into Gen 2.0 concept	100% Complete.
2. **Hydrogel plug chemistry refinement to meet new needs**	• Complete refinement of hydrogel into Gen 2.0 specs • Begin hydrogel packaging verification	80% Complete[a].
3. **Tool design for hydrogel deployment will be refinement to meet new needs**	• Design, build and benchtop verify new Gen 2.0 injector tool • Build out design history file	80% Complete[b].
4. **In vivo validation of Gen 2.0 Hydrogel Plug System**	• Draft and submit animal protocol for in vivo validation of Gen 2.0 system • Produce Gen 2.0 devices for testing	80% Complete[c].
5. **GLP In vitro and in vivo biocompatibility validation**	• Get quotes for GLP biocompatibility testing • Produce samples for GLP testing	80% Complete[d].
6. **Research & Development of Supported Patch Chemistry**	• Propose/Identify best candidates for modifying/improving hydrogel adhesive performance for supported patch	100% Complete.
7. **Research & Development of Unsupported Patch Chemistry**	• Propose/Identify best candidates for modifying/improving hydrogel adhesive performance for unsupported patch	100% Complete.
8. **Research & Development of Unsupported Patch Chemistry**	• No Deliverables scheduled for 2017	100% Complete.

a. Waiting on Gen 2.0 hydrogel from manufacturer. Hydrogel production has been verified. Just waiting on delivery
b. Waiting on Gen 2.0 injector tool SLA from design house. Injector tool design is complete. Component production has started.
c. Waiting on delivery of Gen 2.0 components for starting testing.
d. Waiting on delivery of Gen 2.0 components for starting testing.

3.A. SPECIFIC AIM 1: COLLECT USED NEEDS AND PRODUCT SPECIFICATIONS FROM COMBAT MEDICAL COMMUNITY.

3.A.1. SA1 MAJOR ACTIVITIES.

In 2017, the following key activities were completed with respect to Specific Aim 1:

- Two workshops with military medical personnel were planned to capture user feedback.
- Several ocular sealant injector tools loaded with the current hydrogel adhesive formulations were prepared (>150 units) for hosting an end user workshop
- Draft design requirement questionnaires to capture feedback from users
- Organize end user workshops with military medical personnel
- Build out workshop program and train team for performing workshop
- Execute workshop(s)
- Collect, compile, analyze and interpret feedback from workshops
- Workshop feedback was integrated into the Gen 2.0 hydrogel design
- Workshop feedback was integrated into the Gen 2.0 tool design

3.A.2. SA1 SPECIFIC OBJECTIVES.

The 2017 objectives for Specific Aim 1 were to collect end user feedback on the existing Gen 1.0 hydrogel and injector tool design to help refine engineering design and performance requirements to produce a Gen 2.0 hydrogel and Gen 2.0 injector tool with characteristics preferred by the end users.

Specific Objectives	Month
1.) Draft form to capture active duty medical personnel design criteria + specifications	1
2.) Schedule & hold design review meetings with active duty vision specialists (doctors, medics, nurses).	2-4
3.) Compile key conclusions in Plan of action for Gen 2.0 Redesign. Key areas: user description/area of use description, clinical performance requirements, packaging/transportation requirements, other requirements.	4
4.) Distribute specifications refinement to specific teams for integration into workplan	4

3.A.3 SA1 SIGNIFICANT RESULTS.

We organized and hosted a preliminary user workshop with relevant military medical personnel (ophthalmologists and medics/corpsmen) to capture initial user feedback.

On January 26, 2017, the USC team hosted its first user experience workshop, **Figure 1**, with seven enlisted and retired U.S. military ophthalmologists to capture valuable feedback regarding: a) overall system validity, b) hydrogel performance characteristics, 3) tool performance characteristics, and d) general thoughts on concept and the field of use. The primary objective was to put the prototype system in the hands of potential end users to see if any critical design elements were missing and to also capture qualitative first impressions of the system as a potential product.

Ophthalmologists were able to employ the hydrogel with minimal training.
Each ophthalmologist was given 3 or 4 attempts to deploy the hydrogel on a pig eye model of open globe injury. Only two of the testers were able to successfully deploy the hydrogel on first attempt. However, six of the seven testers were able to successfully deploy the hydrogel on the 2nd or 3rd attempt, **Figure 2**. Some of the challenges with adoption were due to limitations of the tool prototype.

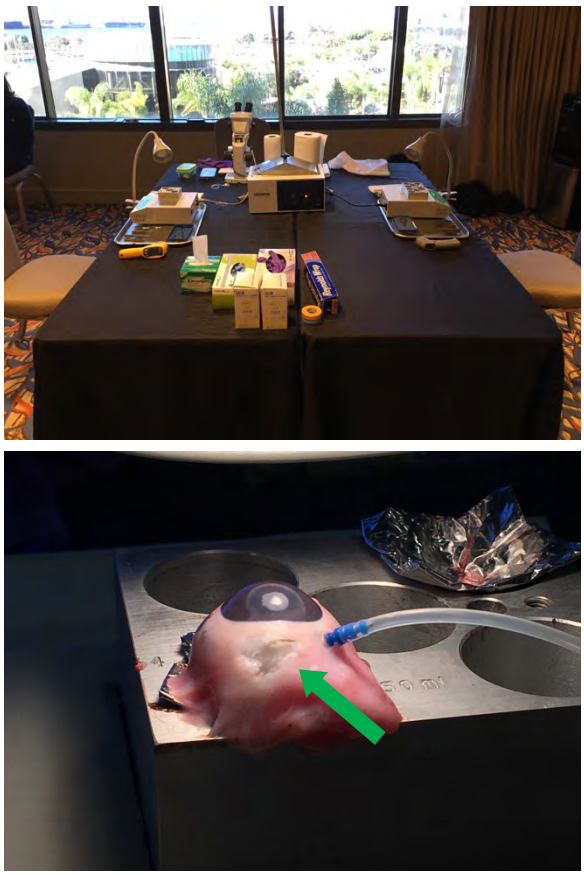


Figure 1. [Top] Photograph of the two workstations (left and right), that were prepared for the user experience workshop. [Bottom] Close up of cannulated pig eye with open globe injury (arrow) visible, prior to testing.

The Sigma hydrogel injection/feel was preferred over the USC formulations that were tested. The sigma hydrogels had the proper viscosity – not too viscous to inhibit flow from the tool and not too runny/fluid that the hydrogel didn't hold its place when injected into the site.

The hydrogel formed a cohesive occlusion at the trauma site. Multiple tests on different sized injuries showed the ability of the hydrogel to conform to the trauma's shape with little modification required. Removal of different hydrogel plugs, post-placement, revealed the shape and volume of the deployed hydrogel, **Figure 3**.

The USC formulation hydrogel began to solidify in the tool's port. During injection, the USC hydrogel showed evidence of solidifying inside the tool; It ejected from the tool in a casing type form, created a folded tube ("spaghetti pile") instead of flowing to fill the opening.

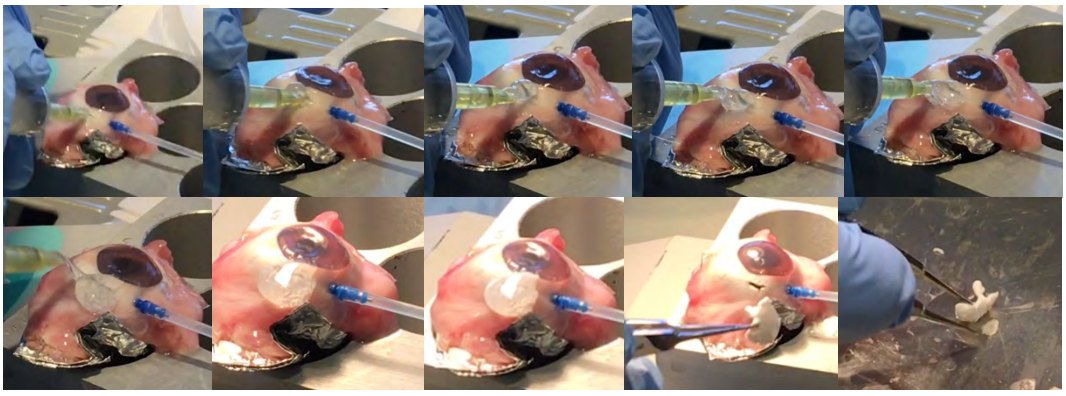


Figure 2. (Beginning Left to Right, top to bottom): Time-lapse photography of hydrogel deployment and subsequent removal for inspection by US Army LTC Marcus Colyer MD (Ophthalmology, Walter Reed Medical Center). Photo series demonstrates usability of system by clinicians with minimal prior training of on using the system.

Figure 3. Examples of three hydrogel plugs removed from open globe sites, after deployment and fixation by the ophthalmology testers at the workshop. These occluding plugs show the cohesion of the hydrogel preparation.

The viscous feel of the hydrogel was highly valued. Comments were made that the fibrin glue based adhesives were "runny" and therefore limited controllability during application.

A syringe style tool is a familiar form factor. Most agreed that the injector-style form factor was a good design because everyone is familiar with these types of instruments and their mode of operation.

Include finger loops on the outer syringe. This was suggested to increase control and manual dexterity during operation of the syringe.

Users considered this a real opportunity to address unmet need. This technology has significant opportunity for mass casualty and far-forward situations where access to full surgical facilities and trained ocular surgeons may be delayed or limited.

Users confirmed that prevention of leakage at the margins of the occluded injury is a good performance metric. Of particular interest for the testers is how the hydrogel performs against injuries with irregular margins and injuries with missing tissues.

Some uses expressed concern about injection design used by non-physicians. Testers commented there might be some risk of causing additional damage to surrounding anatomy in the eye during injection by non-physicians. More work will need to be performed to explore this.

Users suggested that infection prevention would be a significant performance metric. If the hydrogel can prevent bacterial ingress to the posterior chamber, this would be a significant performance advantage. Inclusion of antibiotic drug release would be of interest.

Some concern about epithelial down growth. Epithelial tissue will multiply and expand when not constrained or bounded by other epithelial tissues. In open globe injuries, there is a significant concern that epithelial cells from the conjunctiva and scleral surface will grow and proliferate inside the posterior chamber. Evaluating the hydrogel's ability/inability to prevent this would be a big performance metric to study.

Demonstration of hydrogel stability was requested. Some comments were made regarding whether the hydrogel would be stable enough for transport to severe environments. The team mentioned that extreme temperature exposure is common during transport.

Following the success of this workshop, a follow-on workshop with more participants was planned around the annual US Military Tri-Service Ocular Trauma Workshop held at Walter Reed National Medical Center on May 22-26, 2017.

We organized and hosted a second user workshop with relevant military medical personnel (ophthalmologists and medics/corpsmen) to capture statistically powered user feedback. From May 22-24, 2017, the USC team hosted a user experience workshop at the 2017 annual US Military Tri-Service Ocular Trauma Workshop at the Uniformed Services University at Walter Reed Military Medical Center (Bethesda. MD). The Army's Ophthalmology service organizes an annual ocular trauma workshop to provide continuing medical education for US military ophthalmologists, nurses, medics and others who may encounter, treat or manage casualties presenting with ocular injuries. The 4-day program provides a full day of class-room training followed by three days of laboratory coursework where the registrants are able to practice a variety of examination techniques and intervention techniques on both benchtop and in vivo models of ocular trauma. The USC team was able to coordinate with the workshop directors to secure a laboratory space at the meeting to setup two work stations to allow workshop participants to try the hydrogel sealant system against a benchtop model of ocular trauma over a three-day period.

Of the 140+ registrants for the Military Workshop, 44 came to the USC workstations to evaluate the hydrogel sealant system. Over 140 individuals registered for the Tri-Service Ocular Trauma Workshop, most of which were US military healthcare specialists (ophthalmologists, medics/corpsmen, nurses, scientists), but the registrants also included civilians interested in supplementing their ocular trauma management training.

100% of testers were able to close the ruptured globes by the second attempt. Participants, **Figure 4**, were given two opportunities to try to repair the globe ruptures. The team tracked whether the testers were able to seal the globe on first pass or on the second pass. 43% of testers were able to seal the eye on first attempt. This was only after watching a simple 15min video, followed by providing them with a demo device to get a feel for the viscosity and plunger resistance of the tool.

	Doctors	Medics/ Corpsmen	Other*	Totals per Day
Day 1	7	0	0	**7**
Day 2	15	4	1	**20**
Day 3	15	0	2	**17**
	37	**4**	**3**	**44**

Table 1. Table of all participants that tested the sealant system by job and by day they came to our workstation.

44 individuals participated in the USC workshop evaluation and covered a range of clinical experience, age and backgrounds. 36 ophthalmologists, 1 neurosurgeon, 4 medics/ corpsmen, and 3 research scientists participated in the program, **Table 1**. Stratified by age, **Table 2**, the majority of participants were in group II of the four age groups. The following page includes some photographs of the participants at the workstation testing the hydrogel as well as the USC team managing the workshop. All testers shown are attendees of the tri-service ocular trauma workshop.

Figure 4. Second photo set of ophthalmologists testing the hydrogel sealant (left) and of the USC Team that conducted the 3-day study to evaluate the hydrogel sealant (stud).

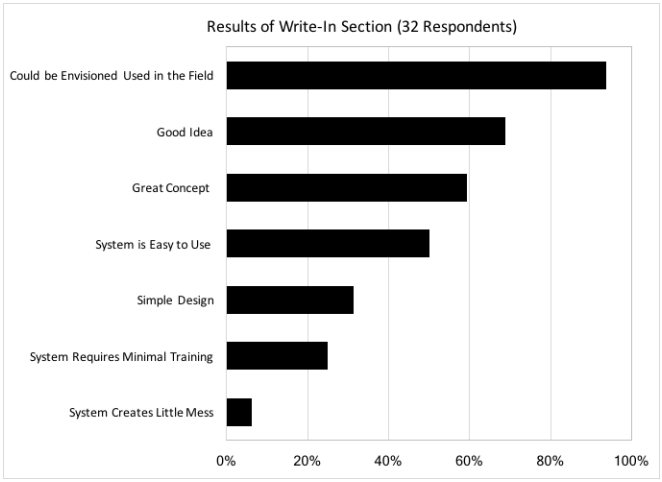
Of the 44 respondents, 32 completed the write-in section of the questionnaire, and 35 respondents completed the multiple-choice section. The majority (59%) of the participants were in the second age bracket (between 31-40), which corresponds with the age group most likely to have been recently deployed. The majority of the Age Group I participants were still in training (e.g. medical school, residency or fellowship) or medics/corpsmen. Participants in Age Groups 3 and 4 were mostly retired from military service but still actively involved in managing open globes. Of the 36 participants, 78% (n=28) reported having firsthand experience with managing open globes. Experience ranged from one open globe per year to as many as 24 globes per year.

Age Breakout for Physicians			
<30	**31-40**	**41-50**	**51+**
8	22	7	7

Table 2. Breakout of participants by age grouping.

The majority of the feedback on the sealant system was positive with some constructive suggestions on how to improve the technology. **Figure 5** plots the results of the open question section where respondents could write in any thoughts in free form. Of the responses collected, 69% of the respondents (n=22) thought the idea of the reversible occlusion for open globes was good idea, with 59% thinking it was great. 31% found the system easy to use with 25% noting that

the system required minimal training. Perhaps most interesting, 94% of the responders (n=30) said they could envision the system being used in the field or in managing combat casualties.



- The system could be envisioned used in the field: **94%**
- The system seems like a good idea: **69%**
- The system is a great concept: **59%**
- The system is easy to use: **50%**
- The system is a simple design: **31%**
- The system requires minimal training: **25%**
- The system creates little mess: **6%**

Figure 5. Results from the write-in section of the questionnaire listed in order of decreasing response percentage.

Of the 35 participants that answered the multiple-choice section, 94% agreed that the system concept seemed feasible and 89% thought it made sense. This was captured on a 5-point scale between strongly agree (5), agree (4), no difference (3) disagree (2) and strongly disagree (1). People selecting 4 or 5 were grouped as "agree" and people selecting 1 or 2 were grouped as "disagree". **Figure 6** plots these results. The numeric values are listed on the following page.

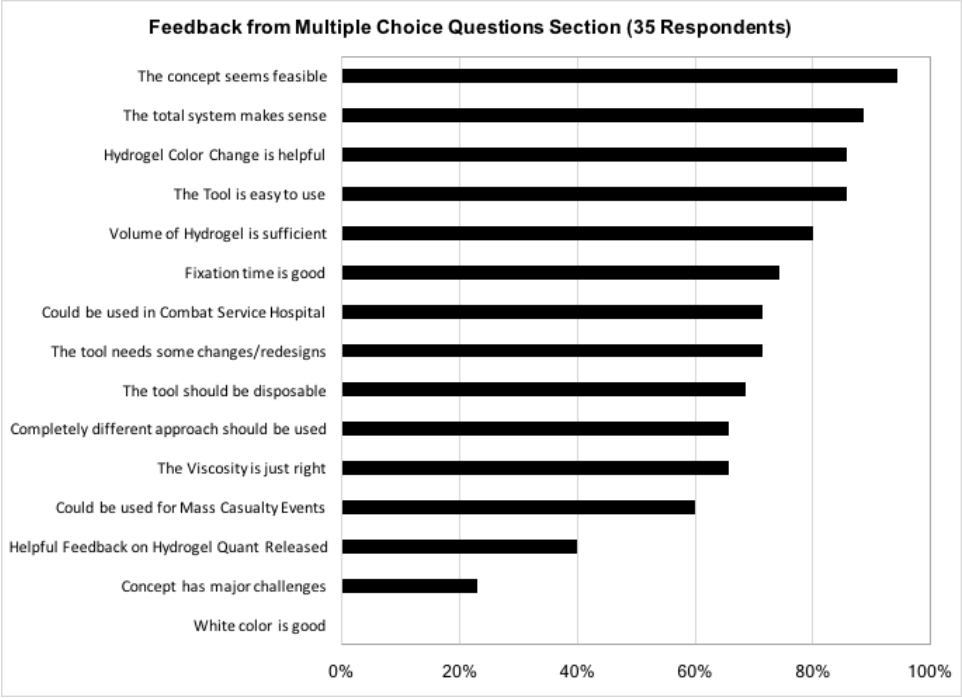


Figure 6. Aggregated participant responses from the multiple-choice section of the questionnaires.

Collectively, the feedback from the clinicians provided good confirmation from a population of relevant end users that the concept we have developed provides a reasonably pragmatic solution to managing posterior segment globe ruptures. We believe some of the redundancies in reported feedback between the write-in and multiple-choice sections help reinforce that the reported feedback was real. These inputs are now being integrated into a final design with plans to begin transition towards clinical safety evaluation.

Summary of actual percent values shown in Figure 6:

- The concept seems feasible: **94%vs. v 0%**
- The total system makes sense: **89% vs. 0%**
- The hydrogel color change is helpful: **86% vs. 0%**
- The tool is easy to use: **86% vs. 9%**
- The volume of hydrogel is sufficient: **80% vs. 9%**
- The hydrogel fixation time is good:**74% vs. 9%**
- The system could be used in a combat support hospital setting: **71% vs. 0%**
- The tool needs some changes/redesigns: **71% vs. 17%**
- The tool should be disposable: **69% vs. 11%**
- A completely different design/approach should be developed for this problem: **66% disagree with statement.**
- The hydrogel viscosity is just right: **66%**
- The system could be used to manage mass casualty events: **60% vs 14%**
- Feedback on hydrogel quantity (audible or visual feedback) would be helpful:**40% vs. 17%**
- The concept has major challenges: **23% vs. 57% disagreeing**
- The white color is good: **No answers**

3.A.4. SA1 OTHER ACHIEVEMENTS

- A protocol and approach to capturing user needs was built from scratch and employed for the user workshops.

SPECIFIC AIM 2: HYDROGEL PLUG CHEMISTRY REFINEMENT TO MEET NEW NEES.

3.B.1. SA3 MAJOR ACTIVITIES.

In 2017, the following key activities were completed with respect to Specific Aim 2:

- Several different hydrogel formulations were prepared and characterized
- Identified hydration percentages that were preferred
- Identified copolymer percentages that were preferred
- Identified polymer molecular weight distributions that were preferred
- Identified vendors to source possible starting materials

3.B.2 **SA2 SPECIFIC OBJECTIVES.**

Specific Objectives	Month
Major Task 1: Identify a better working Gen 2.0 hydrogel	
1.) Place order for, receive, setup and calibrate new rheometer	1
2.) Synthesize new 30% hydrogel for testing	2
3.) Establish benchmark viscosity of the current 30% hydrated co-polymer hydrogel	2
4.) Create a matrix of hydrogel chemistries to be synthesized;Axes: hydrogel molecular weight, other alkyl groups on the acrylate co-monomer, and adhesion enhancing additives.	2
5.) Deliver a lower MW hydrogel formulations that can be prepared <10min with similar viscosity to current solution.	1
Major Task 2: Identify a working strategy for preparing and packaging hydrogel Gen 2.0	
1.) Design, fabricate and validate pressure vessel for hydration storage evaluation.	1-2
2.) Conduct characterization study on Gen 1.0 Hydrogel to establish baseline pressure-hydration relationship	2-3
3.) Synthesize "best candidate" Gen 2.0 hydrogels for pressure-hydration study	3-5
4.) Conduct pressure-hydration study to characterize Gen 2.0 candidates. Identify best performers and report;	2-4

3.B.3 **SA2 SIGNIFICANT RESULTS.**

Approaches to manufacturing medical grade hydrogel have been initiated.

The USC team has begun pursuing ways for GMP manufacturing of its own hydrogel formulations. The team has prioritized 1) investigating different copolymer synthesis routes to identify pathways to viable manufacturing, 2) vetting of contract manufacturing groups which may be able to produce the GMP hydrogel, and 3) initiating steps towards GMP manufacturing of medical grade hydrogel.

Internal work investigating synthesis conditions and parameters have been initiated to look at parameter effects on final product characteristics. One approach used in 2017 to attempt manufacturing of copolymer was to study butyl acrylate (BA) feeding ratio during copolymer synthesis. This approach allowed the LCST of the P(NIPAM-co-BA) to be tuned to help identify

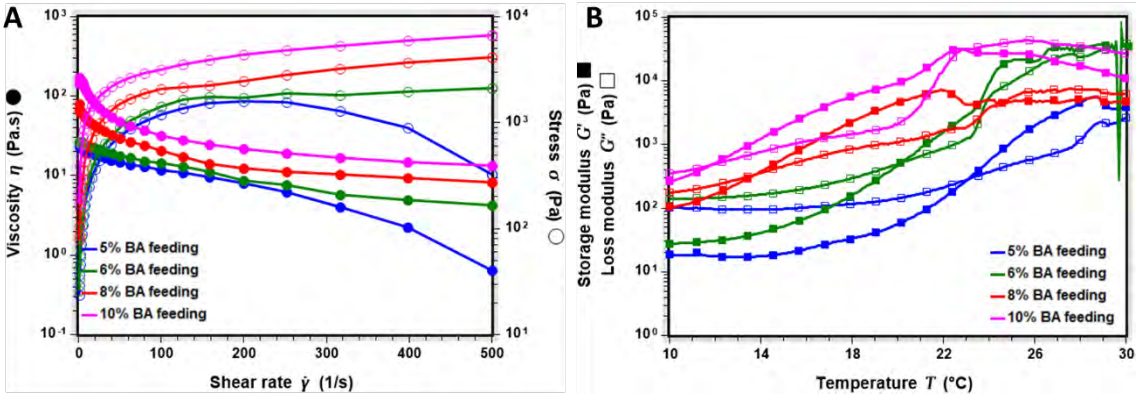


Figure 5. (A) Flow curves of the aqueous P(NIPAM-co-BA) solutions with different BA feeding ratio at 10 ºC. The concentration used was 30%. (B) Oscillatory heating ramp of 30 w/w% aqueous P(NIPAM-co-BA) solutions with different BA feeding ratio. The cross-over point of storage (G') and loss (G'') modulus is defined as the critical gelation temperature (CGT).

the ideal range for the gelation temperature for our ocular application. At a low temperature (below 10 ^{o}C), P(NIPAM-co-BA) can be dissolved in distilled water and forms a homogenous liquid solution. When the temperature is increased, an entropically driven hydrophobic aggregation occurs, and the solution becomes a gel-like solid. The rheological properties of the polymer systems were characterized on our new installed rheometer (DHR-2, TA Instruments), and displayed as **Figure 5**.

A steady flow curve was conducted as a function of the applied shear rate **Figure 5A**. The P(NIPAM-co-BA) solution exhibited yielding and shear-thinning behavior before gelation, which was highly beneficial for injectable gel application. Shear-thinning behavior is consistent with less viscous fluids.

Oscillation temperature ramp was further performed to monitor the gelation behaviors of P(NIPAM-co-BA) solutions, and the critical gelation temperature(CGT) was determined as the crossover point of G' and G''. Representative temperature ramp curves of different P(NIPAM-co-BA) solutions **Figure 5B**, show the incorporation of butyl group in the PNIPAM backbone increased the hydrophobicity of the polymer. This results in a shift of the CGT to lower temperature, and a descending trend with the increased BA feeding ratio **Figure 5B**. Significantly, CGT can be tuned from 11.8 ^{o}C to 23.2 ^{o}C with the fine control of gelation rate.

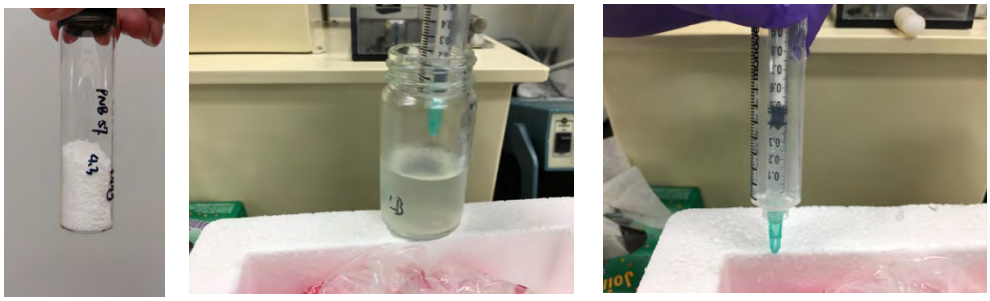


Figure 6. (left) Copolymer hydrogel following synthesis by the Thompson Group exists in a flaky solid state. This was then hydrated into the usable form (center), which was then loaded into the prepared injector tools. (right) Each injector was loaded with between 0.3 and 0.5cc of hydrogel.

***90-100mL of hydrated hydrogel was successfully prepared at USC for the Walter Reed workshop with the desired performance characteristics needed for the sealant to perform properly*.** **Figure 6 (left)** is a photograph of the powder copolymer hydrogel that was synthesized by the Thompson Group for the Walter Reed workshop. This dehydrated form was then mixed with distilled water and stored under refrigeration until ready for loading. Once the injectors were produced, each was individually loaded with between 0.3 and 0.5cc of hydrogel, **Figure 6 (center and right)**. We specifically varied the content to offer testers the ability to evaluate whether more or less hydrogel was preferred in the injector tool.

The USC synthesized hydrogel received significant positive feedback from end users at our workshop. 80% of the respondents to our Walter Reed workshop questionnaire agreed that the quantity of hydrogel was sufficient, with the balance of respondents noting that more hydrogel in the tool might be helpful. 63% found the color change from transparent to white useful, however 86% recommended have a more visible and unnatural color e.g. blue or bright purple.

The USC Team has engaged Polysciences Inc. (Warrington, PA) to provide contract manufacturing services for the hydrogel material.
Polysciences Inc. is an ISO-certified and FDA-registered polymer manufacturing organization which produces a variety of medical grade polymers. The USC team evaluated a number of possible contract manufacturers and decided on Polysciences Inc. based on their proposal, level of experience and total cost estimates.

A kickoff technical meeting was held between the USC Team and the engineering team at Polysciences Inc. USC prepared a brief regarding the approach used for synthesis, the key parameters desired for the finished polymer, e.g. mole fraction of co-monomer content; mean molecular weight; molecular weight distribution; percent hydration; residual monomer tolerances, etc. The objectives set for the program were: 1) to replicate the process used by the Thompson lab and produce a first run of hydrogel at Polysciences; 2) the first run sample will be sent back to USC for characterization and comparison against our preparations; 3) initiate exploration of scalable production approaches.

By the end of 2017 synthesis routes and materials properties details were transferred to Polysciences, acceptance criteria for USC accepting a test batch from Polysciences was set, and work was initiated in polymer R&D at Polysciences. Our development plan followed three stages: Stage 1) replicate the hydrogel production at the same small (<100g scale) as USC. Stage 2) replicate the hydrogel production at an intermediate size production (1-5kg scale). Stage 3) replicate the hydrogel production at large scale without loss of properties.

It was agreed that test samples from Polysciences will be shipped in dry form to USC. The hydrogels would be hydrated by the Thompson team and then subjected to a series of tests to verify hydrogel properties and behavior are consistent with needs. Verification Testing for hydrogels, which would begin in 2018, would consist of the following battery of tests:

A. *Gas Permeation Chromatography (GPC)*. GPC will be used to evaluate molecular weight distribution of the hydrogels. The molecular weight distribution of copolymer in the hydrogel matrix plays a significant role on the mechanical properties of the hydrogel adhesive. To this end, we have characterized the molecular weight distribution of hydrogels and will use these distribution profiles to identify successful synthesis approaches.
B. *Dynamic Viscosity Characterization*. Hydrogel viscosity plays a significant role in the placement, fixation and performance of the hydrogel adhesive. The USC team will perform dynamic viscosity characterization measurements using the viscometer/rheometer as the second criteria for success.
C. *Lower Critical Transition Temperature (LCST)*. If the test samples received from Polysciences successfully meet the first two test criteria, a final bench test will be performed to evaluate the lower critical solution temperature (LCST) for transitioning between hydrophilic and hydrophobic states.
D. *In vivo Performance Testing*. Hydrogel test samples that pass the three bench verification tests, will be scheduled for in vivo performance testing. Ultimately this final test will provide the most useful performance and safety data. In addition to in vivo performance testing, the hydrogels will be sent out for ISO-10993 cytotoxicity testing as well.

3.B.4 **SA2 OTHER ACTIVITIES.**

- No other activities to report for this period.
- We only completed approximately 80% of the deliverables for this objective. Our manufacturing of the hydrogel chemistry was delayed by the opportunity to host the larger end user workshop later in the year. This opportunity allowed us to capture a significant amount of user feedback to integrate into our redesign.

SPECIFIC AIM 3. TOOL DESIGN FOR HYDROGEL DEPLOYMENT WILL BE REFINED TO MEET NEW NEEDS.

3.C.1 **SA3 MAJOR ACTIVITIES.**

In 2017, the following key activities were completed with respect to Specific Aim 3:

- Fabricated over 140 Gen 1.0 units for workshop
- Held workshop to allow end users to use tool and provide design feedback
- Integrated design feedback data into new design requirements
- Translated design requirements to design/manufacturing contractor house for Gen 2.0 concept development.

3.C.2 **SA3 SPECIFIC OBJECTIVES.**

Specific Objectives	Month
1.) Distill feedback from military medical personnel into Gen 2.0 Tool design specs	1
2.) Draft Gen 2.0 tool design and conduct design review to get signoff that specs are met.	1-2
3.) Draft validation testing strategy along with success criteria - get stakeholder signoff	1
4.) Fabricate SLA working prototypes of Gen 2.0 tool for testing	2-3
5.) Conduct benchtop validation to determine if ready for in vivo validation	3-4
6.) Finalize draft Design History File (DHF) for transfer to contract manufacturer	4

3.C.3 **SA3 SIGNIFICANT RESULTS.**

Tool design feedback was captured from end users and integrated into a Gen 2.0 Redesign.

Valuable user feedback regarding the Gen 1.0 tool design, **Figure 7**, was collected at the AUPO and Walter Reed workshops. Observations like premature cannula occlusion and finger slippage from the grip points were examples of some of the characteristics that could be improved. The following significant results were captured regarding tool production and design.

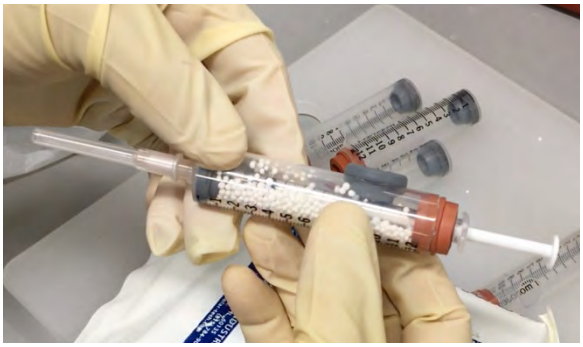


Figure 7. Photograph of loaded prototype Gen 1.0 hydrogel tool prepared for the workshop. Improvements suggested by the workshop participants included finger loops for improved control and manipulation, Luer lock tip for tip placement, and window for seeing hydrogel quantities released.

The USC team fabricated over 150 Gen 1.0 prototype tools for use and testing at bother workshops. The devices were produced using off-the-shelf parts and conventional machine shop facilities. **Figure 8** shows some photographs taken during the production. Overall, this shows the relative ease with which we were able to produce a significant quantity of units with repeatability. Each tool was loaded with between 0.3 and 0.5cc of hydrogel and packaged in groups of 12 in plastic bags for subsequent storage under refrigeration.

Figure 8. Selected photographs showing the production steps for manufacturing the prototype injector tools for the Walter Reed workshop. Circular holes were machined into 12cc syringes to create the outer thermal jackets. Green stopper plugs were machined to create the upper flange through which the 1cc syringes were inserted. 1cc syringe plungers were modified by adding a larger thumb plate for better pressure application during injection. The completed tools were collected, quality checked and packaged in groups of one dozen, for shipment.

All 144 loaded injector tools survived airway shipping without any leakage, breakage or failure in performance. The syringes were packed in three boxes for transport and shipped via overnight airway carrier. Once received in DC, all three boxes' contents were inspected for any visible evidence of leakage or breakage. No injector tool failures were found. After visual inspection, three injectors were randomly selected (one from each shipment box) for performance testing validation. Water was injected into the thermal jacket to initiated cooling and the tools were then used on the benchtop. No qualitative differences in performance were observed compared to in Los Angeles, thus validating they survived transportation.

Regarding the tool design, 86% (n=30) of participants thought the design was easy to use. While 71% did believe that the design could use some minor changes to improve the design, only 23% (n=8) believed major changes were necessary and the same percentage believed there were some major challenges that needed to be addressed in the tool design.

The USC team engaged a medical device product development team and began injector tool Gen 2.0 design. The USC team signed a preliminary agreement with an FDA compliant development house (Symbient PD, Inc.) to being concept build out of the Gen 2.0 injector tool based on the design requirements captured by the team. Design requirements and all known, pore-defined

specifications were aggregated by the USC team for transfer to Symbient in early 2018. The plan will be to generate first concepts in early 2018.

3.C.4 **SA3 OTHER ACHEIVEMENTS.**

- We have developed a viable low cost and repeatable approach to producing our prototype Gen 1.0 devices.
- We only completed approximately 80% of the deliverables for this objective. Our manufacturing of the Gen 2.0 injector tools was delayed by the opportunity to host the larger end user workshop later in the year. This opportunity allowed us to capture a significant amount of user feedback to integrate into our redesign. We are moving forward now with those designs

SPECIFIC AIM 4. IN VIVO VALIDATION OF GEN 2.0 HYDROGEL PLUG SYSTEM.

3.D.1 **SA4 MAJOR ACTIVITIES.**

In 2017, the following key activities were completed with respect to Specific Aim 4:

- Captured user needs feedback from military ophthalmologists and medics to confirm performance needs/requirements are addressed by Gen 2.0 design
- Draft new animal protocol for testing all
- Submit animal protocol to USC IACUC for approval → secure approval
- Submit IACUC-approved animal protocol to ACURO for approval

3.D.2 **SA4 SPECIFIC OBJECTIVES.**

Specific Objectives	Month
1.) Draft and submit ACURO review for approval	2
2.) Produce and sterilize Gen 2.0 products for in vivo validation	5
3.) Perform implantation studies; track tool performance, IOP outcomes, and tissue response	5-7
4.) Perform statistical anslysis of data: 95% CI new system (tool + hydrogel) works effectively	6-8
5.) Compile data for integration into regulatory package	8

3.D.3 **SA4 SIGNIFICANT RESULTS.**

An animal protocol for preliminary safety and performance validation of the Gen 2.0 system (hydrogel sealant and injector tool). Study objectives were reviewed and safety and efficacy objectives were drafted to perform a more comprehensive in vivo risk assessment of the system. The following safety and efficacy objectives were re-affirmed as study objectives:

- **Safety Objectives:**
    - SO1: The hydrogel induces no neurotoxic effects on the retina.
    - SO2: The hydrogel induces no cytotoxic effects on surrounding tissues.
    - SO3: Injection induces no damage to surrounding structures (lens, zonules, retina)
    - SO4: Mean incidence of infection is no greater/less than without intervention.
    - SO5: Probability of epithelial cell migration into the posterior chamber is less with using hydrogel vs. no intervention.
    - SO6: The hydrogel induces no greater incidence of retina detachment vs. no treatment.

- **Efficacy Objectives:**
    - EO1: The hydrogel prevents leakage/dehiscence from the injury site.
    - EO2: The hydrogel provides a statistically significant improvement in IOP
    - EO3: The hydrogel is successfully deployed by the tool on first injection. On second injection.

Feedback from both the team statistician (Dr. Mack) and from the user workshops was used to edit and shape the final draft of the animal protocol. The protocol was submitted to IACUC for review in October and received preliminary approval in early November (**November 03, 2017**). The protocol was submitted to ACURO for review on **December 11,2017** and we anticipate preliminary feedback in January 2018. A copy of the IACUC approved animal protocol is included in the Appendix of this report.

3.D.4 **SA4 OTHER ACHIEVEMENTS.**

- None to report.
- We have been slightly delayed on completion of these objectives by the opportunity to host our expanded end user workshop at Walter Reed Medical Center.

SPECIFIC AIM 5. GLP IN VITRO AND IN VIVO BIOCOMPATIBILITY VALIDATION

3.E.1 **SA5 MAJOR ACTIVITIES**.

In 2017, the following key activities were completed with respect to Specific Aim 5:

- We have reached out to contract research laboratories to request updated price quotes for performing ISO-10993 biocompatibility testing.
- We have begun to draft our in vivo animal protocols modified from the vendor protocols in anticipation of performing GLP in vivo studies.
- Performing GLP testing has been delayed until we can deliver the Gen 2.0 hydrogel and injector tool for safety and performance validation.

3.E.2 **SA5 SPECIFIC OBJECTIVES**.

Specific Objectives	Month
1.) Draft and submit ACURO review for approval	2
2.) Team meeting with contract biocompatibility lab to define SOW and deliverables	4
3.) Produce and sterilize Gen 2.0 hydrogel for biocompatibility; delivery to biocompatibility lab	5
4.) Conduct MEM elution/extraction cytotoxicity testing on selected "best candidate" chemistries.	6
5.) Results review - select formulations for in vivo biocompatibility testing; Fabricate/deliver	7
6.) Conduct GLP *in vivo* biocomp testing in accordance with ISO-10993 protocol standards.	8-12
7.) Statistical analysis of data and interpretation	11-12
8.) Results review and next steps determination	12

3.E.3 **SA5 SIGNIFICANT RESULTS.**

Nothing significant to report other than the activities described.

3.E.4 **SA5 OTHER ACHIEVEMENTS.**

- Draft of a biocompatibility study for future submission to IACUC and ACURO has been initiated, but is awaiting review with regulatory consultant.
- Our kick off of animal studies was delayed by the user workshop – which allowed us to collect more design validation information.

SPECIFIC AIM 6. RESEARCH & DEVELOPMENT OF SUPPORTED PATCH CHEMISTRY.

3.F.1 **SA6 MAJOR ACTIVITES.**

In 2017, the following key activities were completed with respect to Specific Aim 6:

- Chemistry modification of the adhesive hydrogel was investigated to improve hydrogel-to-tissue adhesion. This approach would enhance topical "band-aid" style intervention designs.
- A variety of different approaches to adhesion modification of the hydrogel have been investigated this year including: 1) Modifiation with gelatin and chitosan, 2) Modification by addition of Guanadino-Propionic Acid (GPA); and 3) Modification by addition of tannic acid.
- Benchtop characterization of the adhesion of GPA-modified and tannic acid modified hydrogels to cadaveric porcine sclera.

3.F.2 **SA6 SPECIFIC OBJECTIVE.**

Specific Objectives	Month
1.) Identify (on-paper) a series of "best candidates" hydrogel-on-substrate patches to investigate	9
2.) Fabricate patches (different cross-linking densities and anchoring strategies) for testing	9-12
3.) Perform benchtop verification (Bose, IOP testing)	12-15

3.F.3 **SA6 SIGNIFICANT RESULTS.**

The Thompson group has been focusing supported patch development efforts on ways to improve the adhesion between the hydrogel surface and the scleral tissue surface. Surface chemistry characterization of the sclera has been performed and found that it is predominantly negatively charged. As a results the Thompson group has been exploring new formulations that incorporate positively charged polymers (adhesive polymer). The positively charged polymer helps to promote tight adhesion to the tissue surface.

As shown in **Figure 9**, four (4) different commercial polymers were selected as the adhesive agent, including gelatin, chitosan, branched polyethyleneimine (PEI) and poly (allylamine hydrochloride) (PAH). Functionalization with amine groups guarantees stronger interaction with ocular mucins, and could also conjugate with P(NIPAM-AA) copolymers via the carbo-di-imide crosslinker chemistry.

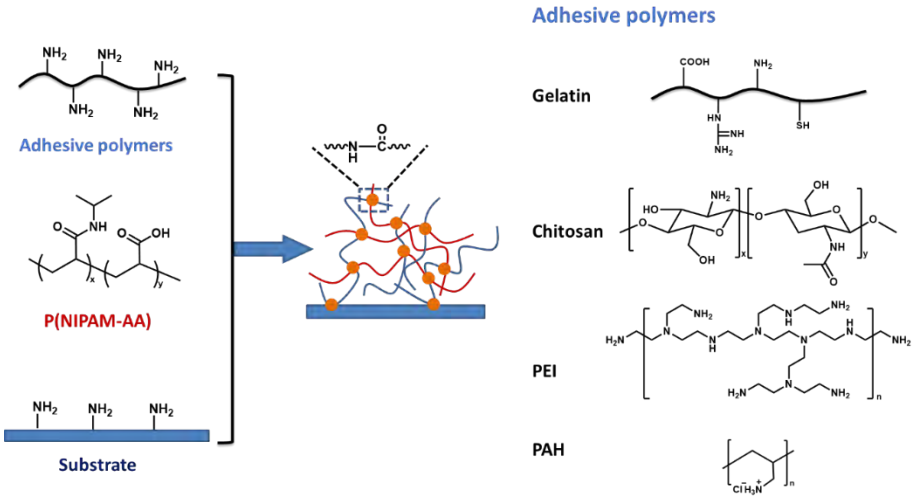


Figure 9. The formation of PNIPAM/adhesive polymer cross-linked networks for supported hydrogel patch application.

Different synthesis routes were explored to produce these new copolymer formulations for adhesion and mechanical property characterization. The PNIPAM copolymer functionalized with carboxyl groups was first synthesized via RAFT copolymerization of NIPAM and acrylic acid (AA). The carboxyl groups were used to form amide bonds with the amino groups of adhesive polymers in the presence of a water-soluble carbo-di-imide. The experimental protocols were similar for different polymers except gelatin. As a thermoreversible gel, gelatin is soluble in water at temperatures above T_{gel} and forms reversible physical gel below T_{gel}, which is just opposite to PNIPAM. Therefore, the polymer concentration was exactly controlled to keep all the polymers in the solvated state at room temperature. Oscillatory time sweep can be used to check the cross-linking reaction. As shown in **Figure 10A**, compared with the simple blending of P(NIPAM-AA) and gelatin, both the storage modulus(G') and loss modulus(G'') increased after addition of EDC·HCl and overnight incubation, and the solution displayed a predominantly elastic character, with G' higher than G" over all the investigated time range, which was the direct proof of the successful crosslinking of the two components. And the cross-linked P(NIPAM-AA)/gelatin

hydrogel showed an elastin behavior all through the measured temperature range, and the gel was further stiffened above the LCST of PNIPAM, while the G' of the uncross-linked P(NIPAM-AA)/gelatin mixture was steadily decreased above the LCST of PNIPAM, and appeared as an opaque solution at high temperature (**Figure 10B**).

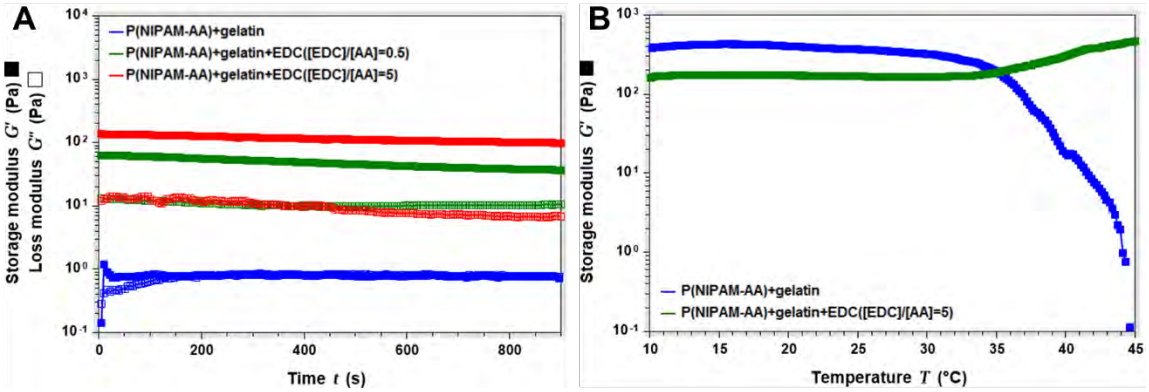


Figure 10. (A) Oscillatory time sweep of P(NIPAM-AA)/gelatin mixture at 28 °C after overnight incubation with different EDC feeding ratios. (B) Comparison of the temperature dependence of storage modulus (G') of P(NIPAM-AA)/gelatin mixture with and without EDC.

The Thompson group has demonstrated, on the benchtop, the ability to improve topical adhesion of hydrogel formulations to scleral tissues with the use of tannic acid (TA) . For this quarter investigation focused on modifying both the hydrogel and the sceral tissue surfaces with tannic acid. Tannic acid (TA, **Figure 11A**) is a polyphenol compound that is found ubiquitously in virtually all plant species. TA shows multiple intermolecular interactions with polymers, and acts as a molecular glue. TA is being explored as an intermediate layer, with its dual-interactions with both tissue surface molecules and polymers, and a tough adhesive system could be formulated while reserving the thermo-reversibility of polymers.

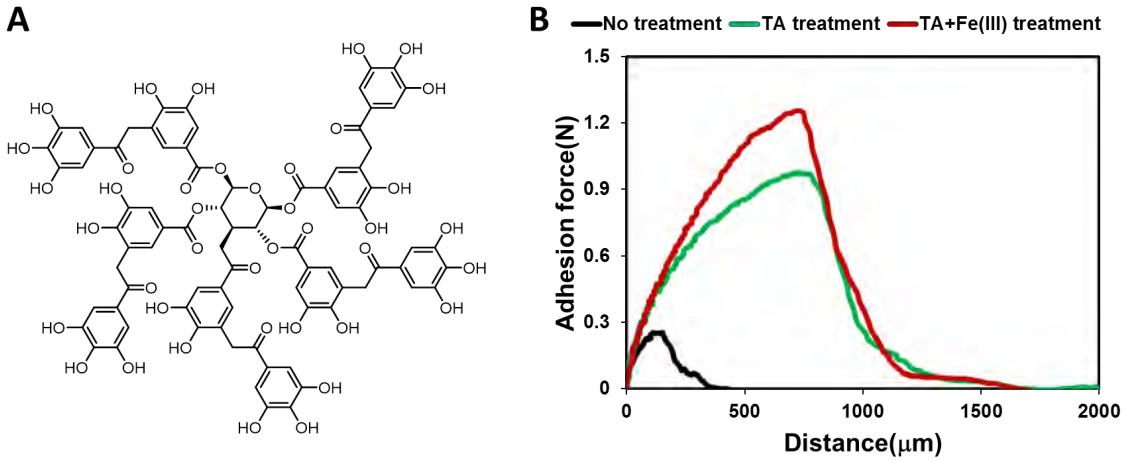


Figure 11. (A) The chemical structure of tannic acid (TA). (B) The adhesive strength of 30 w/w% aqueous RP P(NIPAM-co-BA) solution to porcine sclera at 32 °C with different TA treatments.

Preliminary benchtop measures were performed to assess the adhesive strength of the copolymers modified with TA. Linear pull adhesion tension testing for maximum adhesion strength, **Figure 11B**, suggest the force is greatly enhanced after TA treatment of the porcine sclera, and the strength

is further increased using coordination complexes of TA and Fe(III) ions. These are preliminary measures and we will perform multiple measures to extract a mean value.

3.F.4 **SA6 OTHER ACHIEVMENTS.**

- None to report.

SPECIFIC AIM 7. RESEARCH & DEVELOPMENT OF UNSUPPORTED PATCH CHEMISTRY.

3.G.1 **SA7 MAJOR ACTIVITIES.**

In 2017, the following key activities were completed with respect to Specific Aim 7:

- Hydrogel cohesion was characterized by molding uniaxial test pieces and performing conventional pull tests.
- Rheological characterization of hydrogels modified with different additives were also performed to look at cohesion and viscosity.
- Data was compiled and analyzed to help steer next steps towards topical adhesion of the hydrogel sealants.

3.G.2 **SA7 SPECIFIC OBJECTIVES.**

- No 2017 objectives/deliverables.

3.G.3 **SA7 SIGNIFICANT RESULTS.**

The Thompson Group has been characterizing cohesive properties of molded, unsupported patches of hydrogel. The proposed hydrogel patches must exhibit both strong adhesion to the ocular surface as well as strong cohesion of the hydrogel patch to prevent internal tearing under stress. Adhesion testing to scleral tissues has been performed using our Bose uniaxial pull tester (mentioned in previous reports), **Figure 12, left**.

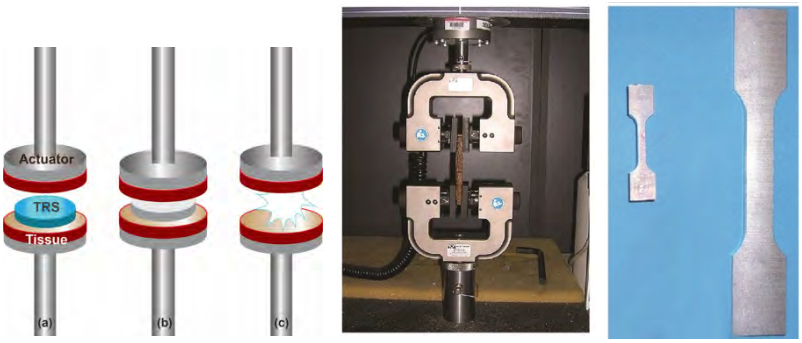


Figure 12. (left): Schematic illustrating the uniaxial adhesion testing approach that has been used to test hydrogel adhesion. (center): Photograph of the Instron 5942 which is being used to test hydrogel cohesion. (right): Photograph of a representative metal alloy test piece typically used to in the Intron tester.

Hydrogel cohesion characterization required modifying a different but more conventional uniaxial pull tester setup is being employed, **Figure 12, center**. An Instron 5942 tensile tester was modified to perform these tests. Test pieces for these studies have a characteristic "dogbone" shape, **Figure 12, right**, which allows the test fixtures to grasp the two ends of the test piece and also allows the test stress to be focused through the narrow central portion.

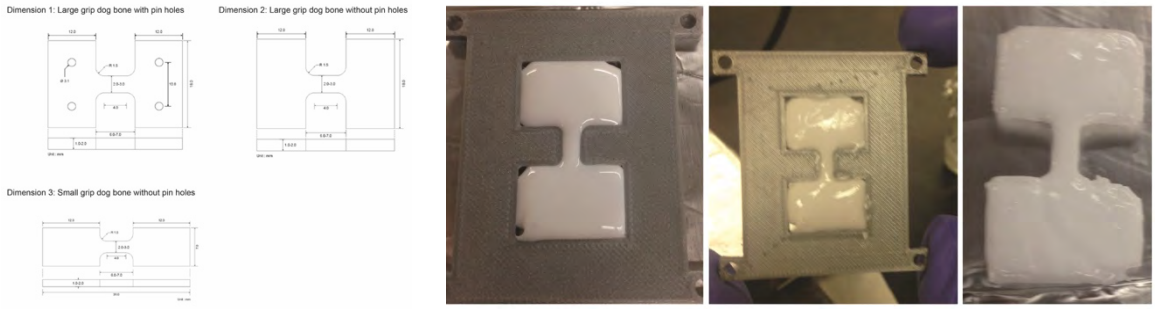


Figure 13. (left): Computer drafts of custom designed hydrogel test mold used to form test pieces for cohesion testing. (middle two photos): Photographs of the hydrogel placed in the mold and heated to solidify enough for removal. (right): Molded hydrogel test piece released from the metal mold.

Custom molds were fabricated to produce hydrogel test pieces for the Instron system. In order to perform these uniaxial cohesion studies an aluminum mold was machined to produce hydrogel test pieces in the dogbone shape required, **Figure 13**. Liquid hydrogel samples were shifted to the solid phase (32°C) using a hot plate and were detached from a metal mold of the same dimensions as the previous plastic mold. Samples were fixed to substrates with cyanoacrylate at each wing of the butterfly, and clamped at the substrate. The solid samples were stretched at a constant rate of 1mm/min under controlled temperature, **Figure 14**. Young's modulus was determined as the slope of the linear region of the stress-strain corresponding to 0%-10% strain.

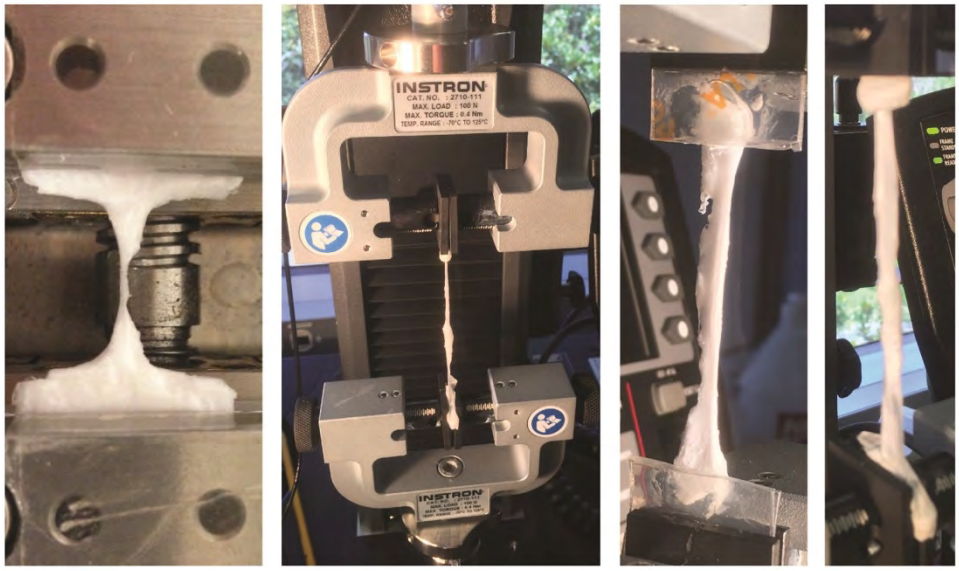
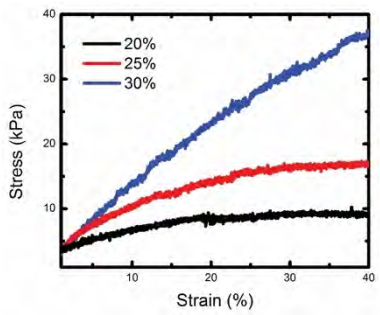
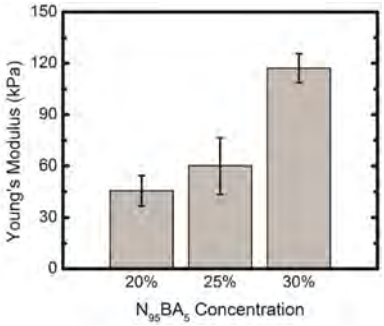


Figure 14. Time-lapse photograph series (left to right) of a hydrogel test piece being pulled in a test run using the Instron. Stretching without breaking is a qualitative indicator of some cohesion.

Tensile stress-strain characterization and tensile modulus quantification of N95BA5 hydrogel (20%, 25% and 30% w/w) at T = 32°C were performed. The stiffness and resilience of the 30% (w/w) $N_{95}BA_5$, as the model material, was evaluated by casting it into tension test sample shapes then performing standard tensile tests on these samples. Tensile stress-strain curves illustrated a positive correlation between TRS concentration and compressive moduli. The results revealed that the mechanical properties of the developed hydrogel can be readily tuned by changing the hydrogel concentration. Elastic moduli of $N_{95}BA_5$, **Figure 15 (left)** was characterized as a function of the concentration (20%, 25%, 30%). TRS tensile stress-strain curves followed a similar trend as compression, with increasing copolymer concentration resulting in higher Young's modulus, **Figure 15 (center)**. 30% TRS hydrogels were found to have elastic moduli of 117 kPa, representing improved stretching capacity compared to 60 kPa (25%) to 45 kPa (20%), **Figure 15 (right).**





	20%	25%	30%
Young's Modulus	35.8	53.9	119.3
	48.1	47.6	107.7
	53.1	78.8	124.4
Average	**45.7**	**60.1**	**117.1**
Standard Deviation	**8.9**	**16.5**	**8.5**

Figure 15. (left): Representative stress-strain curves for hydrogels of different percent hydrations (20%, 25% and 30%). (middle): Calculated Young's modulus values from stress-strain curve data. (right): Table of compiled data from test series performed.

The Thompson group has also begun rheological test measures to characterize the complex viscosity across a range of temperatures to compare additive effects. Relatively small concentrations of GPA or RGDS increased the sample viscosity and slightly decreasing the critical temperature. These tests were performed with 0.5% strain, 15 radians per second oscillation frequency, and a temperature ramp rate of 5 °C /min to prevent any significant dehydration of the sample before test completion.

The rheological properties of the copolymer hydrogels were improved by addition of specific quantities of selected polymer additives. The Thompson group has explored tissue adhesion improvement using guanadinoproprionic acid (GpA) as well as the use of RGD-sequence adhesion proteins. **Figure 16** shows a series of four different rheological characterization experiments performed with our base hydrogel copolymer but functionalized with different concentrations of GpA or RGD proteins. In these plots, the complex viscosity is plotted as a function of temperature. Of the samples prepared, we see the greatest improvement in viscosity from the hydrogels that were modified with the highest concentration of GpA. These chemistries demonstrated the greatest increase in viscosity at the transition temperature (T > 28C).

A series of USC-synthesized hydrogels with GpA additive have been prepared, packaged and sterilized for performing some in vivo performance and safety testing. This will be one of the sample preparations that will be evaluated in our first in vivo tests for this project.

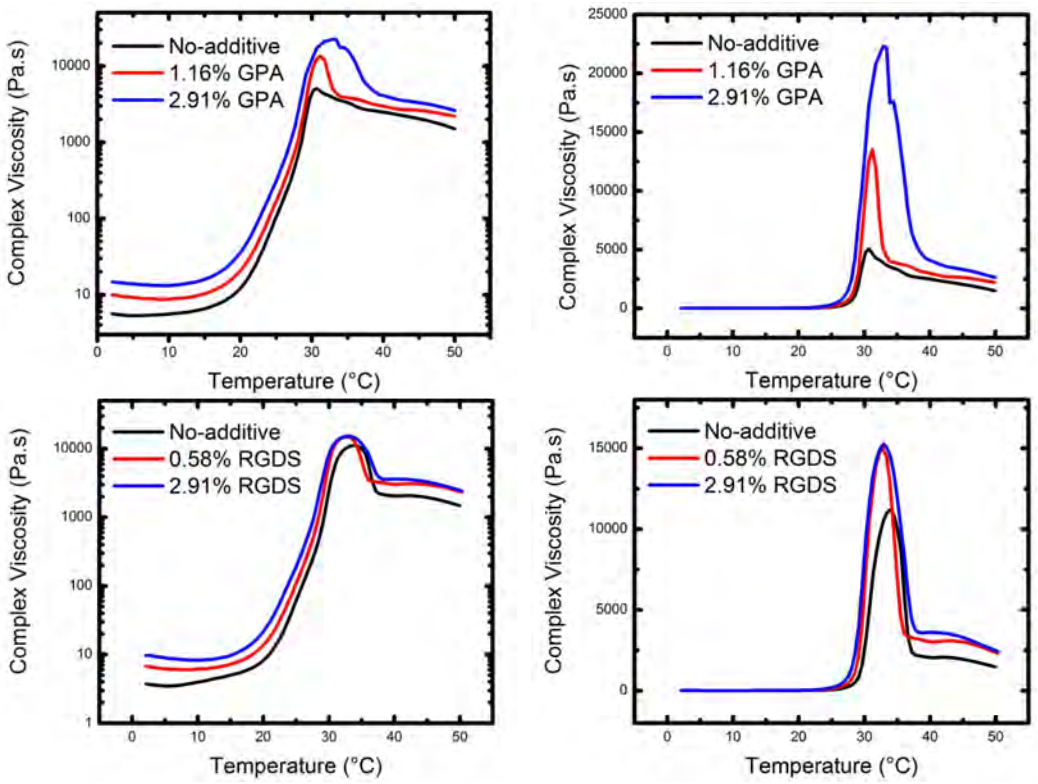


Figure 16. Complex viscosity vs. temperature curves plotted on y-log scale (left plots) and same data plotted in a y-linear scale (right plots) for hydrogel sealant modified with GPA (top row) and RGDS (bottom row) additives. In all cases, increasing quantities of additive result in an increased measured viscosity across the temperature curves.

3.G.4 **SA7 OTHER ACHIEVEMENTS.**

- A custom rig for performing pull tests was developed which uses custom molded test pieces.
- Uniaxial pull test protocols and rheological characterization testing protocols have been developed to evaluate the unsupported hydrogel chemistries.

SPECIFIC AIM 8: RESULTS OF THE PRECIEEDING SPECIFIC AIMS WILL BE COMPILED INTO A DRAFT IDE PACKAGE FOLLOWED BY IDE PACKAGE AT THE END OF THE PROGRAM.

3.H.1 **SA8 MAJOR ACTIVITIES.**

In 2017, the following key activities were completed with respect to Specific Aim 8:

- Began accumulating latest regulatory filings related to hydrogels and ocular medical devices
- A preliminary meeting with our regulatory consultant was held in 2017 to update on technical progress and discuss strategies for regulatory filing. It was agreed that predicate

devices and materials safety data would be collected and reviewed with the consultant once the new Gen 2.0 designs were more developed.

3.H.2 **SA8 SPECIFIC OBJETIVES.**

- No 2017 objectives/deliverables.

3.H.3 **SA8 SIGNIFICANT RESULTS.**

No significant results to report.

3.H.4 **SA8 OTHER ACHIEVEMENTS.**

- None. Nothing to report.

3.I.1 **TRAINING & PROFESSIONAL DEVELOPMENT OPPORTUNITIES.**

In 2017, the following graduate students made significant contributions to the efforts performed:

- Niki Bayat (Chemistry, PhD candidate): Ms. Bayat has learned about hydrogel chemistry synthesis and has been exposed to early stage medical product development. By helping with the planning and execution of the end user workshops she has learned more about how user needs are captured, analyzed and converted into design requirements.
- Roby Menefee (Chemistry, MS candidate): Mr. Menefee has also learned about hydrogel chemistry synthesis and has been exposed to early stage medical product development. Mr. Menefee has learned about medical product development by participation in our Walter Reed end user workshop.

3.J.1 **RESULTS DISSEMINATION.**

- An oral presentation of results to date was given at the August 2017 MHSRS meeting. The presentation reported on our bench top testing and in vivo rest results. A pdf of this presentation is attached in the Appendix.
- A manuscript regarding our findings for this project was submitted in Fall 2017 and accepted for publication in Science Translational Medicine December 2017. A copy of this article is included in the Appendix of this report.

3.K.1 **PLANS FOR NEXT REPORTING PERIOD.**

The following table lists all deliverables for completion by the 2018 annual report.

Specific Aim	Deliverables due by Q4 2018
1. **Collect used needs and product specifications from combat medical community**	• No new deliverables.
2. **Hydrogel plug chemistry refinement to meet new needs**	• Synthesize Gen 2.0 hydrogels using FDA-compliant approach • Complete storage/package stability testing on hydrogels
3. **Tool design for hydrogel deployment will be refinement to meet new needs**	• Complete manufacturing of Gen 2.0 samples for validation testing. • Draft Design history file for regulatory package & tracking
4. **In vivo validation of Gen 2.0 Hydrogel Plug System**	• Complete in vivo performance and safety validation of Gen 2.0 injector tool and hydrogel adhesive. (non-GLP testing)
5. **GLP In vitro and in vivo biocompatibility validation**	• Setup and perform GLP cytotoxicity and biocompatibility testing on selected Gen 2.0 concepts.
6. **Research & Development of Supported Patch Chemistry**	• Produce supported patch chemistry hydrogels for testing • Perform bench testing for direct adhesion to cadaveric porcine sclera • Perform in vivo testing for topical adhesive closure of ocular trauma
7. **Research & Development of Unsupported Patch Chemistry**	• Produce unsupported patch chemistry hydrogels for testing • Perform bench testing for direct adhesion to cadaveric porcine sclera • Perform in vivo testing for topical adhesive closure of ocular trauma
8. **Compile a draft IDE package for submission to FDA for pre-submission meeting feedback.**	• Compile all supporting information, documentation and data for preparation of draft IDE package. • Review results with regulatory consultant to identify missing materials and build plan for completing draft • Draft and review clinical trial documentation for pilot safety study. • Submit with questions to FDA for pre-submission meeting

4. IMPACT:

4.A. IMPACT ON PRINCIPAL DISCIPLINES DEVELOPMENT.

This project is beginning to make an impact in two different disciplines: 1) hydrogel chemistries 2) ocular trauma management.

Hydrogel Chemistries. The Thompson Group has conducted significant effort in synthesizing a variety of different base hydrogel chemistries coupled with different additives, and then performed characterization studies on those formulations. This library of information is helping to expand the greater community's understanding of thermo-responsive hydrogels and how their properties can be modulated.

- *Distinctive Contributions*: Our first publication has shed some light on how different synthesis approaches have produced hydrogels with difference properties. Follow-on papers will be added to this knowledge base.
- *Major Accomplishments & Innovations*: We have identified which chemistries are optimal for implantation and occlusion of ocular trauma.
- *Successes*: We have published a preliminary paper and will be producing at least one more paper that focuses on relating performance characteristics to chemistries.
- *Changes in Behaviors/Practices*. None.

Ocular Trauma Management. The workshop we have held with military medical personnel who have treated or seen combat-related ocular trauma have been open to the idea of rethinking how ocular trauma is managed through the entire role of care.

- *Distinctive Contributions*: We have proposed a novel approach to managing ocular trauma which expedites preliminary intervention and allows intervention under significantly austere environments.
- *Major Accomplishments & Innovations*: We have allowed end users to test our concept and many have been receptive to the idea of rethinking how ocular trauma is managed.
- *Successes*: We have advanced our preliminary concept an approach into a 2nd stage of refinement and development.
- *Changes in Behaviors/Practices*. None to date.

4.B. IMPACT ON OTHER DISCPLINES.

Nothing to Report but we have received inquiries about employing our adhesive sealant for other (non-ophthalmologic) penetrating injuries management.

4.C. TECHNOLOGY TRANSFER IMPACT.

Medical device technology commercialization is a primary motivation of this project. To date we have begun to build an intellectual property portfolio which is already under examination by the USPTO. We have begun to talk with larger medical device manufacturers about our development efforts. The USC team plans to leverage our significant experience in medical technology commercialization to begin to translate this technology from the university into a commercial entity, once we have de-risked the technology to an acceptable level.

4.D. **SOCIETAL IMPACT.**

The USC Team believes this project, if successful, may have at least one impact on society beyond the scientific and technological contributions.

Impact on Trauma Management Paradigms. If successful, this technology would enable healthcare to rethink how healthcare is administered for ocular trauma patients and in particular in mass casualty events where time, resources and facilities may be heavily strained. This technology allows interventions to be performed in austere environments which would free up resources and facilities to manage other life threatening conditions.

5. CHANGES/PROBLEMS:

CHANGES IN APPROACH.
Nothing to Report.

ACTUAL/ANTICIPATED PROBLEMS/DELAYS.
In 2017, we experienced one minor unanticipated delay as a result of an unanticipated opportunity to conduct the larger user workshop at Walter Reed with clinical end users. This workshop was scheduled and held in May 2017, which delayed our design refinement by several months. However, it is noted that this opportunity was invaluable in providing us significant end user feedback.

SIGNIFICANT CHANGES TO EXPENDITURES.
To date we have not had significant changes to increase expenditures. However, we anticipate there may be a challenge with project costs for materials and supplies in 2018 as we further advance the design development and manufacturing for the Gen 2.0 devices.

SIGNIFICANT CHANGES IN USE OF VERTERBRATE ANIMALS.
None.

HUMAN SUBJECTS.
Not applicable.

SIGNIFICANT CHANGES TO CARE AND USE OF VERTRATE ANIMALS.
We have begun the process of securing approvals for our proposed in vivo safety and performance assessment studies (rabbit model). No significant changes to report.

CHANGES TO BIOHAZARD OR SELECT AGENTS.
Not Applicable.

6. PRODUCTS:

PUBLICATIONS, CONFERENCE PAPERS, PRESENTATIONS.

JOURNAL PUBLICATIONS.

Bayat N, Zhang Y, Falabella P, Menefee R, Whalen JJ, 3rd, Humayun MS, Thompson ME. **A Reversible Thermoresponsive Sealant for Temporary Closure of Ocular Trauma**. *Sci Transl. Med*. 2017 Dec 6; 9(419).

ONE-TIME PUBLICATIONS (BOOKS, OTHER PERIODICALS).

Nothing to report.

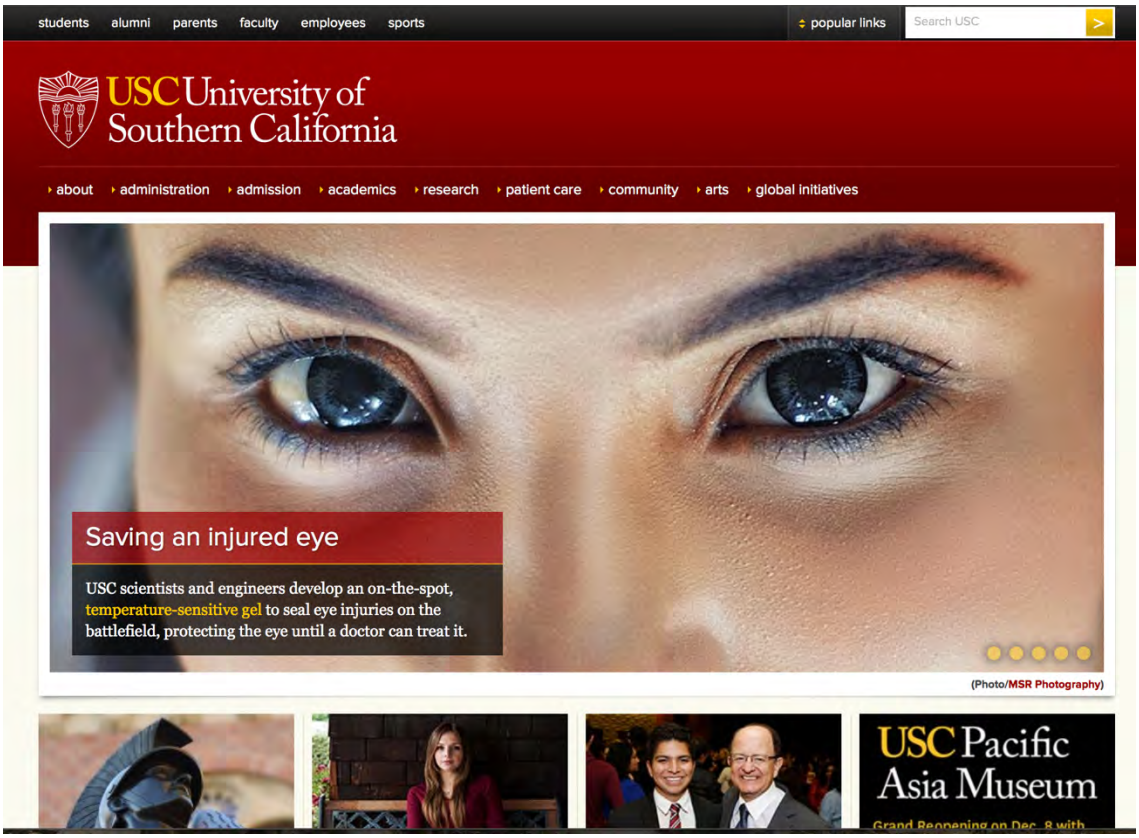
OTHER PUBLICATIONS, CONFERENCE PAPERS AND PRESENTATIONS.

Oral presentation at MHSRS 2017 Orlando FL. 28 August 2017. John J. Whalen III, Niki Bayat, Yi Zhang, Paulo Falabella, Andrew N. Bartynski, Mark E. Thompson, Mark S. Humayun. "Sutureless Hydrogel Ocular Repair System (SHORS): A Reversible Thermo-Responsive Sealant for Temporary Closure of Ocular Trauma."

Oral presentation at 2017 Tech Connect. The team was extended an invitation to present at the 2017 Tech Connect Meeting on Ocular Trauma Intervention Technologies. A presentation was given by Jack Whalen (USC Ophthalmology) and manufacturing partner representative, Alice Lai (Camtek LLC).

WEBSITE(S) OR OTHER INTERNET SITE(S)

The team's Science Translational Medicine article led to several follow-up press notifications published on a variety of websites, including a front page piece on the University of Southern California's main homepage (http://www.usc.edu). The additional webpage publications are listed in the hyperlinks that follow.



List of subsequent press releases that were generated from the release of this publication are listed below:

December 6, 2017: USC university press release:
https://news.usc.edu/132588/a-new-portable-gel-that-could-save-an-injured-eye/

December 6, 2017: USC Keck School of medicine press release:
http://hscnews.usc.edu/a-new-portable-gel-that-could-save-an-injured-eye/
December 6, 2017: Ureka Alert press release:
https://eurekalert.org/pub_releases/2017-12/uosc-cei120417.php

December 6, 2017 on HealthDay: https://consumer.healthday.com/eye-care-information-13/eye-and-vision-problem-news-295/coming-soon-a-gel-that-could-help-save-soldiers-eyes-729155.html

December 6, 2017 on RT: https://www.rt.com/news/412216-reversible-superglue-eye-injuries/

December 6, 2017: Newswise press release:
https://www.newswise.com/articles/combating-eye-injuries-with-a-reversible-superglue-seal

December 7, 2017 on Optometry Today: https://www.aop.org.uk/ot/science-and-vision/research/2017/12/07/sealing-battlefield-injuries-with-reversible-superglue

December 7, 2017 on IFL Science: http://www.iflscience.com/health-and-medicine/injecting-smart-glue-could-be-a-temporary-fix-for-severe-eyeball-injury-on-the-battlefield/

December 7, 2017 on Science Alert: http://www.sciencealert.com/superglue-gel-protects-wounded-eyeballs-saving-sight-vision

December 7, 2017 on Med India: http://www.medindia.net/news/healthinfocus/gel-to-fix-eye-injuries-175310-1.htm

December 7, 2017 on Med Gadget: https://www.medgadget.com/2017/12/novel-transforming-hydrogel-treats-emergency-battlefield-eye-injuries.html

December 8, 2017 on Alphr: http://www.alphr.com/science/1007922/superglue-for-your-eyeballs-is-now-a-thing

December 8, 2017 on Digital Trends: https://www.digitaltrends.com/cool-tech/injured-eyeballs-superglue/

December 8, 2017 on Press Trust of India: https://economictimes.indiatimes.com/magazines/panache/future-of-health-this-temperature-sensitive-gel-treatment-could-seal-eye-injuries/articleshow/61981314.cms

December 8, 2017 on Zee News: http://zeenews.india.com/health/scientists-just-developed-a-gel-to-treat-eye-injuries-2064219

December 9, 2017 on International Business Times: http://www.ibtimes.co.uk/eyeball-superglue-that-washes-off-water-can-prevent-vision-loss-1650711

December 9, 2017: CBS This Morning (aired on more than 150 CBS affiliate stations): http://mms.tveyes.com/PlaybackPortal.aspx?SavedEditID=51cad940-ad20-4ef9-9205-070b3d197969 (also on YouTube at timepoint 3:50: https://www.youtube.com/watch?v=qTysmI-Ptqs)

December 12, 2017 on Eye Smart: https://eyesmart.com.au/newsarticle/5420-novel-gel-developed-to-treat-eye-injuries

TECHNOLOGIES OR TECHNIQUES.

Our efforts continue to drive delivery of a medical device technology to temporarily treat ocular trauma. This will be a medical device and the details have been explained in this report.

INVENTIONS, PATENT APPLICATIONS OR LICENSES.

A provisional patent has been formally converted to a patent application.

OTHER PRODUCTS.

Nothing to report.

7. PARTICIPANTS & OTHER COLLABORATING ORGANIZATIONS

INDIVIDUALS ON THE PROJECT.

Name:	*Mark S. Humayun MD PhD*
Project Role:	*Principal Investigator, Professor*
Research Identifier:	*ORCID: 0000-0002-5830-5208*
Nearest person month worked:	*1*
Contribution to Project:	*Dr. Humayun provides clinical expertise and guidance and overall oversight on activities of the project.*
Funding Support:	*N/A.*
Name:	*Mark E. Thompson PhD*
Project Role:	*Co-Investigator, Professor*
Research Identifier:	*ORCID: 0000-0002-7764-4096*
Nearest person month worked:	*1*
Contribution to Project:	*Dr. Thompson is responsible*
Funding Support:	*N/A.*
Name:	*John J. Whalen PhD*
Project Role:	*Co-Investigator, Assistant Professor*
Research Identifier:	*ORCID: 0000-0002-5202-4747*
Nearest person month worked:	*6*
Contribution to Project:	*Dr. Whalen is responsible for project management of the project, setup and execution of the in vivo testing.*
Funding Support:	*N/A.*
Name:	*Yi Zang PhD*
Project Role:	*Post-doctoral Research Assistant*
Research Identifier:	*N/A*
Nearest person month worked:	*3*
Contribution to Project:	*Dr. Zang has constructed all of the injector tool prototypes and loaded them with hydrogel. He has*
Funding Support:	*N/A*
Name:	*Bin Li PhD*
Project Role:	*Post-doctoral Research Assistant*
Research Identifier:	*N/A*
Nearest person month worked:	*6*
Contribution to Project:	*Dr. Li has synthesized many of the new additive hydrogel chemistries and has been performing many of the characterization studies on these preparations.*
Funding Support:	*N/A.*
Name:	*Niki Bayat*
Project Role:	*Graduate Student*
Research Identifier:	*ORCID: 0000-0002-6070-2392*
Nearest person month worked:	*12*
Contribution to Project:	*Ms. Bayat performed the majority of hydrogel synthesis and materials characterization.*
Funding Support:	*N/A.*

CHANGES IN ACTIVE SUPPORT FOR PI OR KEY PERESONNEL.
Nothing to report.

PARTNERING ORGANIZATIONS.
No other organizations. Nothing to report.

8. SPECIAL REPORTING REQUIREMENTS

QUAD CHART.
Please see the attached 2017 annual quad chart in the Appendix.

9. APPENDICES:

CONTENTS:
Appendix 2: AUPO Workshop Summary with Photos.
Appendix 3: Walter Reed Tri-Service Workshop Summary with Photos.
Appendix 4: Walter Reed Workshop questionnaire.
Appendix 5: MHSRS 2017 Presentation.
Appendix 6: Science Translational Medicine (Publication)
Appendix 7: Patent Applications
Appendix 1: Annual Report Quad Chart 2017

Appendix 1. AUPO Meeting Workshop: First Ocular Hydrogel Sealant End User Testing

The workshop was held at the 2017 AUPO (Association of the University Professors of Ophthalmology) Meeting in San Diego CA. The USC team (Niki Bayat, Yi Zhang, Andrew Bartynski, Juan Carlos Martinez, Jack Whalen) setup the workshop in a small conference room with space for an introductory presentation and a space for bench testing the hydrogels. Doctors which participated in this first workshop are listed in a registration table found in the Appendix of this report.

The workshop itself ran from 11am through 3pm. A brief 20-minute review of the technology, its mode of function and the testing protocol. This was followed by having each ophthalmologist spend approximately 30 minutes at one of the workstations practicing using the hydrogel on a benchtop model of open globe injury.

Figure 2. (Left, from L to R). US Navy CPT. Frank Bishop MD at workstation 1 preparing for first attempt to use hydrogel. USC's Juan Carlos Martinez MD, preparing pig eyes for next round of testing. USC's Yi Zhang PhD, loading and checking next hydrogel tool for testing. US Navy CDR Todd Mondzelewski MD, setup at workstation 2. US Army LTC Marcus Colyer MD, operating the hydrogel tool in a test to occlude an open globe injury.

Two work stations were setup in the meeting room, **Figure 1**, and a cannulated pig eye which had undergone a partial vitrectomy and had at least a 3mm full-thickness scleral wound, was setup on a heated stage. The ophthalmologists were given a hydrogel loaded tool and asked to attempt to occlude the penetrating injury by injecting the hydrogel through the injury, **Figure 2**. A member of the USC team provided support through each test, talking each ophthalmologist through the procedure. Each ophthalmologist was given at least three attempts to occlude the injury. Observations, comments and video were all recorded.

Our Gen 1.0 Hydrogel was Tested Against Aggressive Trauma Challenges.

In some instances, the ophthalmologists elected to modify the penetrating injury by: increasing laceration length; introducing stellate cross cuts through the tissue; or removing portions of sclera to have tissues missing, **Figure 3**.

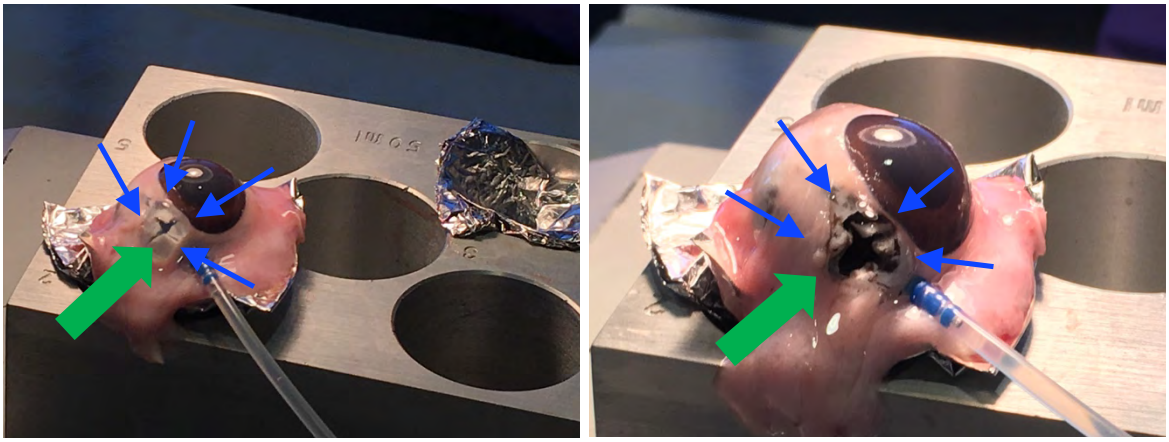


Figure 3. Examples of two modified open globe test challenges immediately following hydrogel deployment, in situ. Left shows a photograph of a stellate injury (green arrow) immediately post-placement of the hydrogel. Right shows an open globe injury with missing scleral tissue and irregular margins (green arrow). The edge of the hydrogel adhesive can be seen in both photographs (blue small arrows)

Appendix 2. Walter Reed End User Workshop for Ocular Hydrogel Sealant

Test Protocol. The test protocol was very similar to the test exercise we conducted at the AUPO Meeting in San Diego (January). An enucleated pig eye was cannulated to allow warm saline infusion. A partial vitrectomy was performed on each eye prior to cannulation. When ready. The eye was placed on a heated stage and any type of full thickness rupture was created on the eye **Figure 1(left)** to create the trauma simulation model. At a minimum, a 5mm incision was created. Stellate ruptures and open holes with missing tissue were created with end to end sizes as large as 20mm, **Figure 1 (center)**. Digital video and photographs of the procedures were captured for many of the tests, **Figure 1 (right)**.

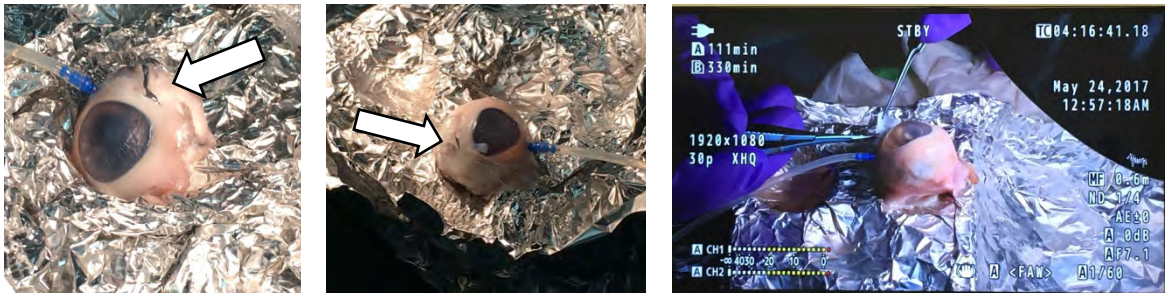
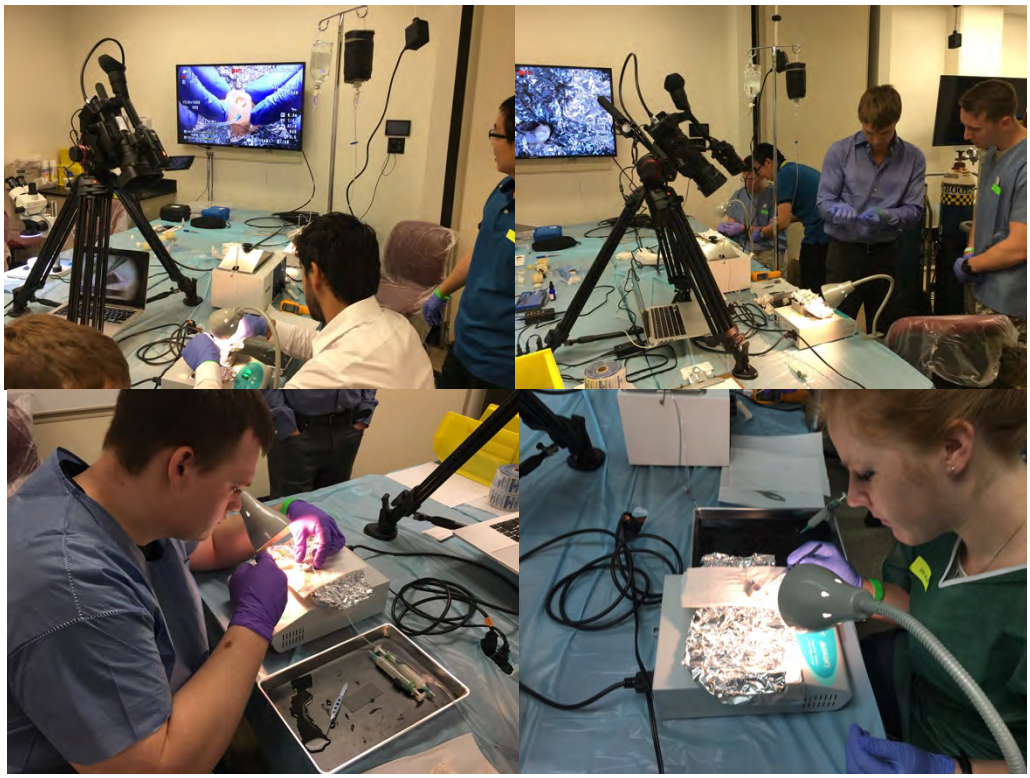


Figure 1. (left) Example of large laceration (L = 2.0cm nonlinear) eye trauma model. **(center)** Example of smaller laceration (L = 5mm, linear). **(right)** Video capture of ocular trauma intervention procedures.

Participants were first shown a video of the system being used to occlude a full thickness scleral laceration in a rabbit eye in vivo, to get a feel for how the system works and how it should be used. The participant was then given a demo tool to practice deploying the hydrogel on the benchtop to get a feel for the hydrogel viscosity and flow as well as the plunger resistance of the tool. Lastly, the participant was shown the location of the laceration on the pig eye and given a fully activated tool ready for sealant placement.

Data Collection via Questionnaire. A 4-page questionnaire was drafted to captured user feedback on each individual. The questionnaire had four sections: 1) a demographics/information section; 2) an open answer section for participants to write in answers; 3) a multiple-choice section with a series of statement of which the participants were asked to select on an a five-point scale of disagree to agree with the statement; 4) a section tracking the results of the actual injection test. Our goal was to capture the participants' levels of experience, age and exposure to managing open globes and use this to stratify data from the other sections to look for any trends.



Appendix 3: Walter Reed Workshop questionnaire.

Appendix 4: MHSRS 2017 Presentation.

Appendix 5: Science Translational Medicine (Publication)

Appendix 6: Patent Applications

Appendix 7: Annual Report Quad Chart 2017

Tester Information for Stratification	Date & Time:	
Name or Initials:		
Contact info (Optional):		
Age Bracket:	☐ ≤30 ☐ 31-40 ☐ 41- 50 ☐ 51+	
Level of Training (res, fellow, etc):		
Field Experience (Y/N):		
# of Years/Months of Field Experience:		
Experience with Managing Open Globes (Y/N):		
# of Open Globes seen in any given time period:		
(e.g. 5 globes in 3 mo.'s, 12 globes in a year)		

Following Presentation – Preliminary Thoughts if any (without using)

Prior to Use:					
1. General Thoughts on System Approach/ Design/ Mode of Use					
2. General Thoughts on Tool Design					
3. General Thoughts on Hydrogel					
4. General thoughts on managing open Globes					
5. Could you envision using this in the field?					
6. Other notes/Comments					

Testing Protocol

1. **Run 1 (TEST):** Allow user to perform test run on using previous user's eye and hydrogel (if any). Thus allowing an introduction to how system is used.
2. **Run 2 (ACTUAL):** Prepare new syringe tool and eye with predetermined incision size 3-5mm. Allow tester to perform independently.
3. **Run 3 (ACTUAL):** Either allow tester to repeat previous exercise OR allow them to try a more aggressive trauma (larger, stellate, corneal).

1. Learning Run: (reuse old eye from previoius)		
Trauma description: Successful Deployment: Time given to fix hydrogel: Amount of hydrogel used:	Comments made by user:	
2. Run #2 – Real Test		
Linear incision Size: Successful Deployment: Time given to fix hydrogel: Amount of hydrogel used:	Leakage observed?	Max IOP (bag height)
3. Run #3 – Real Test		
Trauma description: Successful Deployment: Time given to fix hydrogel: Amount of hydrogel used:	Leakage observed?	Max IOP (bag height)
4. Additional Notes:		

Post-Testing Questions

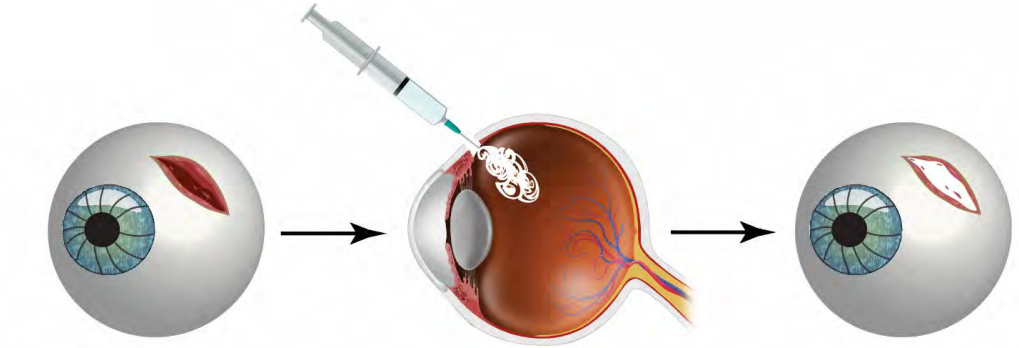
	Totally Disagree	Disagree Somewhat	Either Way	Agree Somewhat	Completely Agree
General Impressions/Thoughts	1	2	3	4	5
1. The total system (hydrogel and tool) makes sense.					
2. The concepts mode-of-use seems feasible.					
3. The concept has major challenges.					
4. Where would you envision this technology being used					
5. Other comments/notes/observations:					

	Totally Disagree	Disagree Somewhat	Either Way	Agree Somewhat	Completely Agree
Tool Design	1	2	3	4	5
1. The tool design is easy use in the mode described.					
2. The tool design requires some changes/modifications.					
3. The tool should use a completely different approach/design?					
4. Disposable vs. Reusable?					
5. Is feedback on the quantity of hydrogel released, required? Helpful?					
6. Other notes/comments/observations:					

	Totally Disagree	Disagree Somewhat	Either Way	Agree Somewhat	Completely Agree
Hydrogel Design	1	2	3	4	5
A. Viscosity. Too viscous? Too runny? Just right?					
B. The transparency change in helpful/suitable for the application					
C. Color. The transparent/white color is acceptable?					
D. The timing speed of fixation is good?					
E. The quantity of hydrogel is sufficient.					
Other Comments/notes/observations					

	Totally Disagree	Disagree Somewhat	Either Way	Agree Somewhat	Completely Agree
Locations for Use/Persons Using System	1	2	3	4	5
1. Could this system be used in a CSH environment?					
2. Could this system be used in mass casualty situation to triage?					
3. Besides an ocular specialist, who else might be able to use this? ER doctor? Any physician? Any surgeon? Nurse? Medic?					
4. How does this compare to other approaches?					
5. Final Thoughts					

USC Institute for Biomedical Therapeutics



Sutureless Hydrogel Ocular Repair System (SHORS): A Reversible Thermo-Responsive Sealant for Temporary Closure of Ocular Trauma

Niki Bayat[1,4], **John J. Whalen III**[2,3], Yi Zhang[2], Paulo Falabella[2], Andrew N. Bartynski[4]
Mark E. Thompson[1,5], Mark S. Humayun[2,3]

1. Department of Chemical Engineering, University of Southern California, Los Angeles, California 90089, United States.
2. USC Eye Institute. Department of Ophthalmology. Keck School of Medicine. University of Southern California
3. USC Institute for Biomedical Therapeutics. Department of Ophthalmology. Keck School of Medicine. University of Southern California
4. AesculaTech, Inc.
5. Department of Chemistry. Dornsife College of Arts, Science and Letters. University of Southern California

J. Jack Whalen III PhD
Asst. Prof. of Ophthalmology Research
USCRoski Eye Institue. USC Institue for Biomedical Therapeutics.
USCKeck School of Medicine.
jjwhalen@med.usc.edu

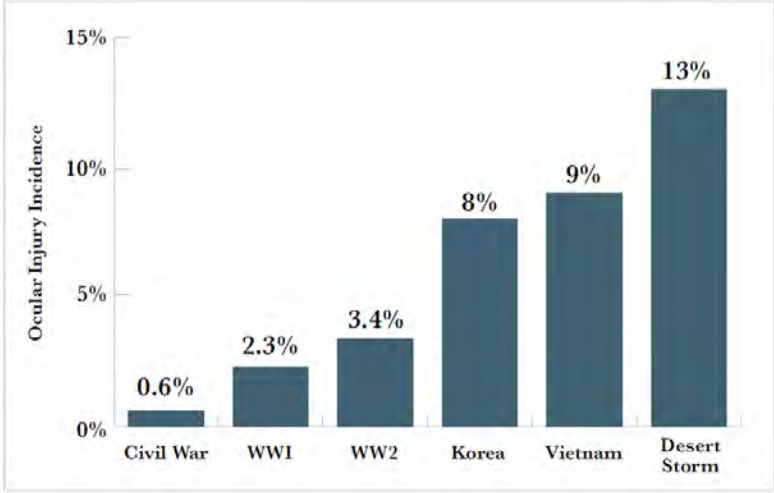
Disclosure: The presenter is a co-inventor of this technology and would stand to benefit financially from any products developed from the technology presented here. The presenter has no financial relationships to disclose.

Motivation

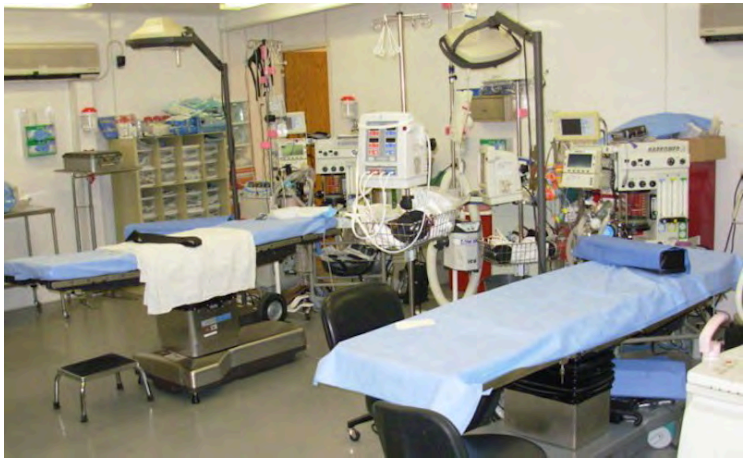
1. **Ocular trauma Incidence has increased.**
   - Use of IEDs
   - Improved mortality rates

2. **Visual Acuity (VA) outcomes decreases with delayed intervention.**
   - Eyes left untreated for longer lose more VA

3. **Looking for Novel Approaches to accelerate intervention.**
   - Temporarily occlude the eye
   - Reduce intervention time
   - Eliminates need for microsurgical equipment

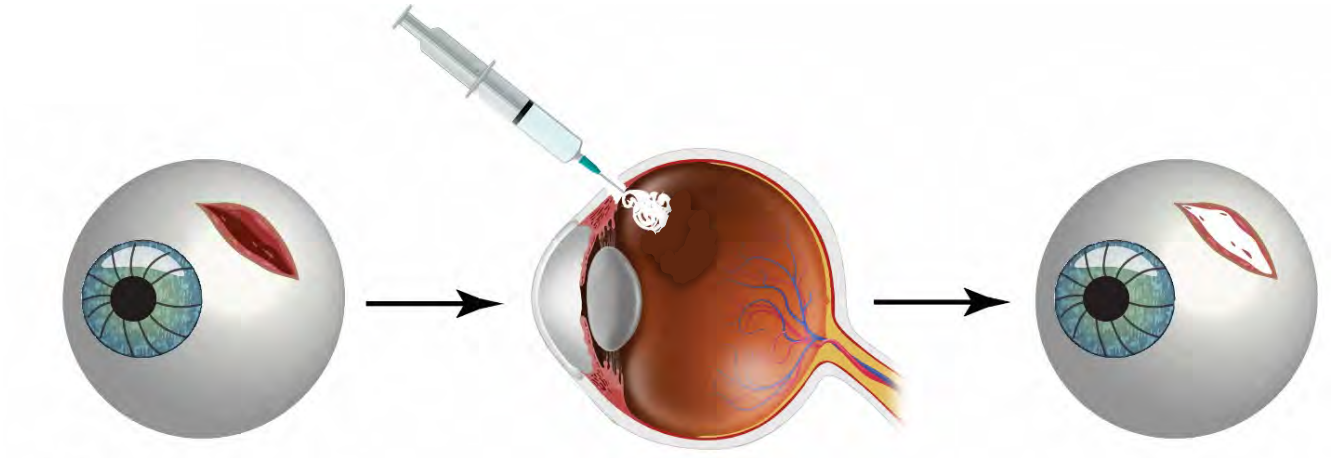


Comparison of incidence of US armed forces ocular injury as a percentage of total combat casualties vs. Military campaign showing the stead rise over time (from Cho and Savitsky, 2012)



Combat Support Hospital (Iraq)

Concept: A reversibly deployable sealant for temporarily occlusion of ruptured globe.

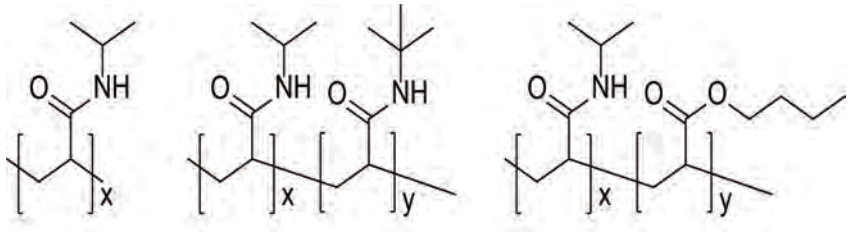


Bayat et al.

- Easy deployment (e.g. injectable, no microscope required).
- Occludes injuries of various sizes (including missing tissues & stellate injuries).
- Rapid fixation.
- Can be used in austere environments.
- Reversible placement. No additional trauma to tissues.
- Temporary.
- Easily produced, packaged, transported.

Enabling Technologies

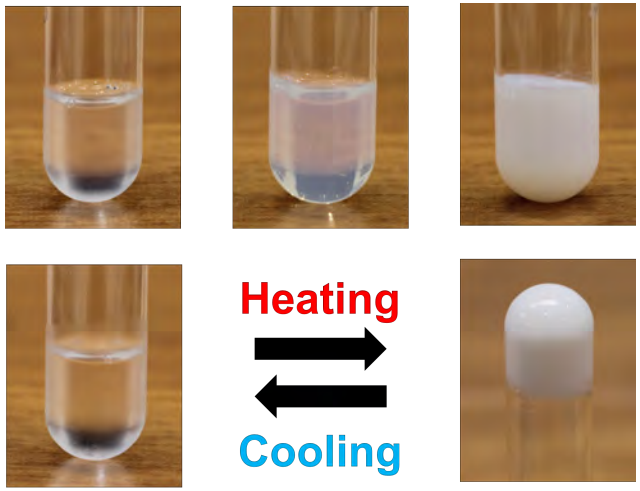
Thermally-Tuned Thermoresponsive Hydrogel.



PNIPAM

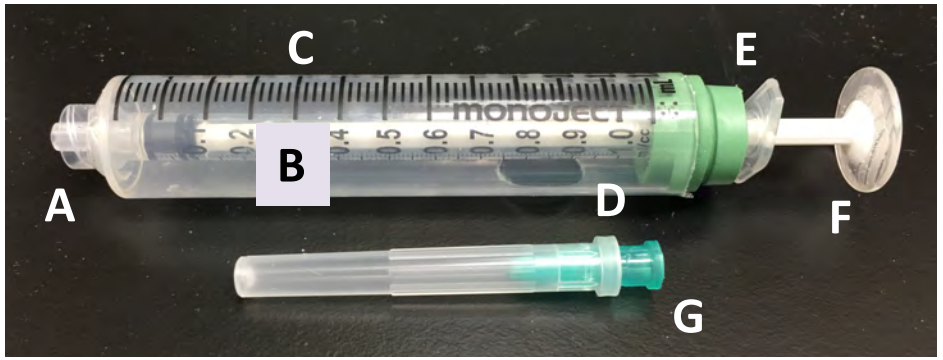
NIPAM-co-N-tert-Butylacrylamide (N_xNT_y)

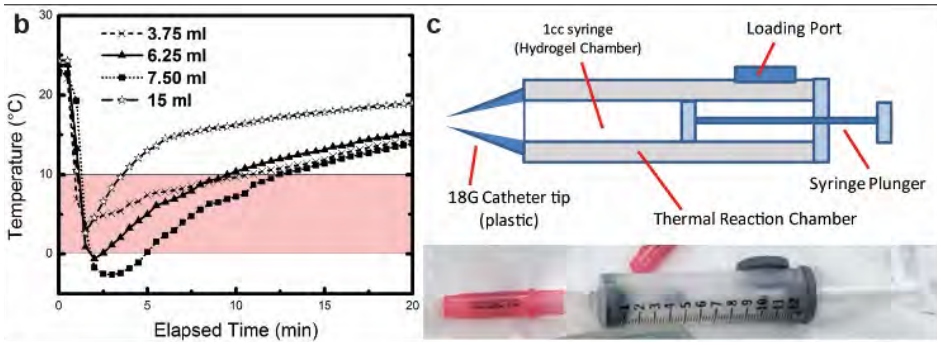
NIPAM-co-Butylacrylate (N_xBA_y)



Bayat et al.

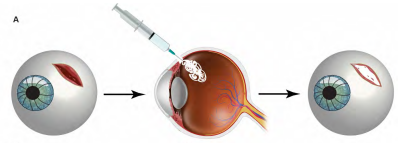
Disposable, Ready-to-Use Applicator Tool





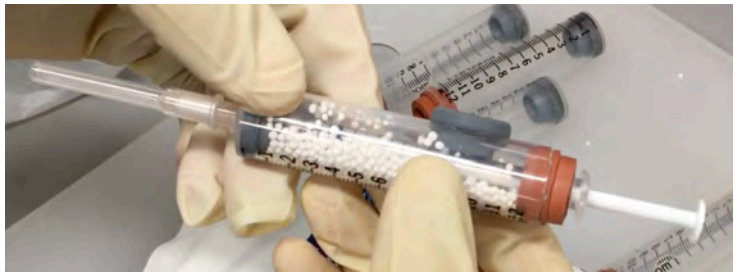
Humayun & Whalen Group

System Mode of Operation

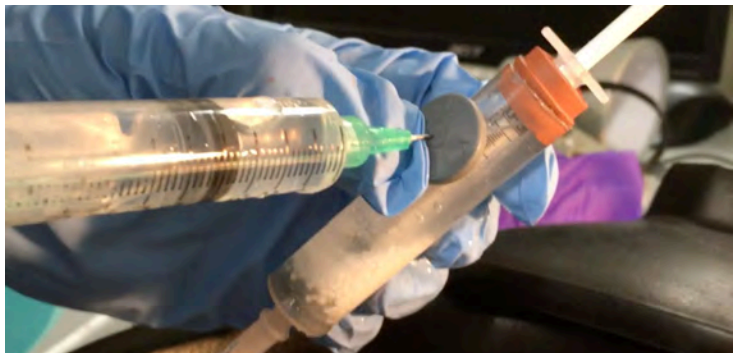


Injector Tool Operation

Injector tool as packaged

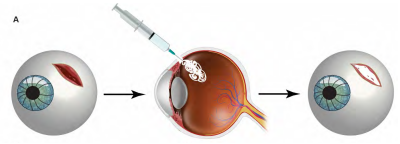


Cooling activation by H_2O

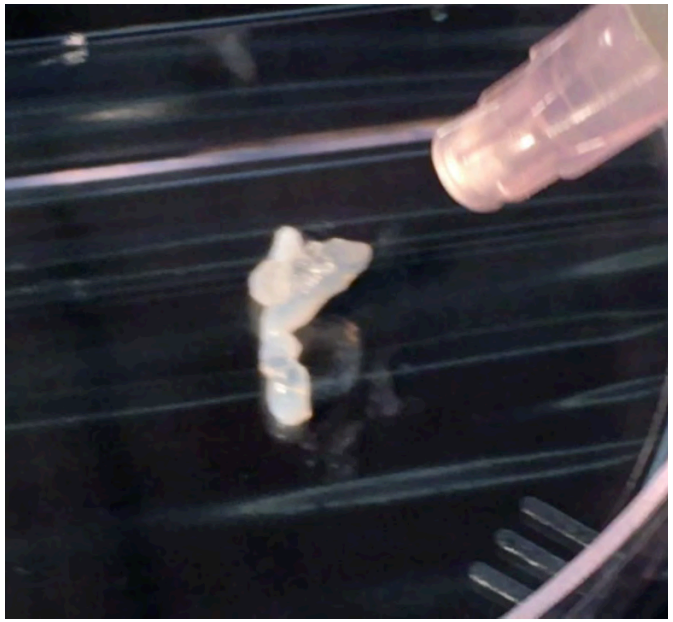


Cooling activated after 30s.

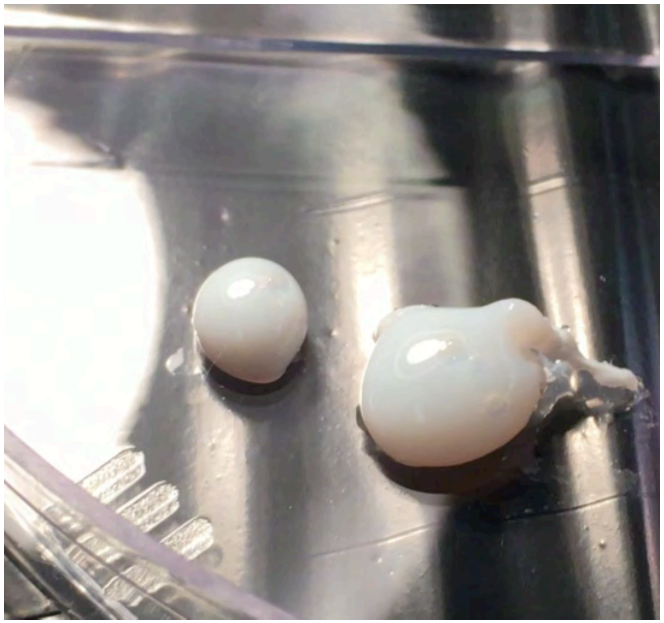
System Mode of Operation



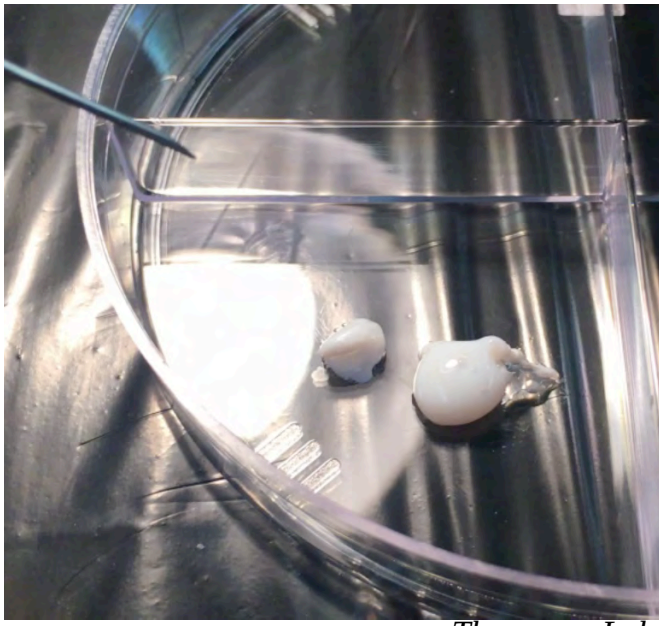
Hydrogel Deployment



Hydrogel Fixation

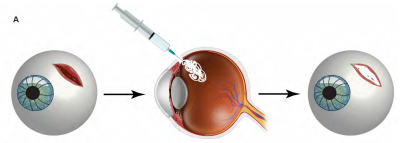


Hydrogel Reversibility



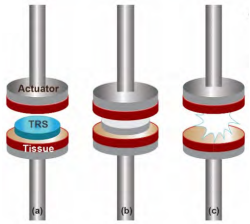
Thompson Lab

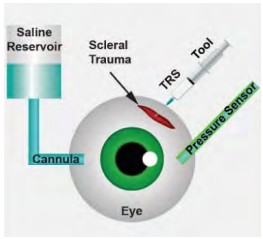
Development Approach

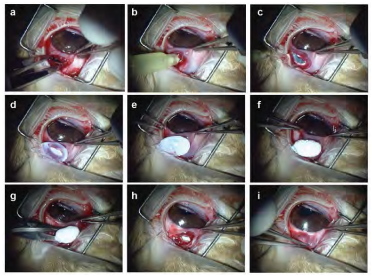


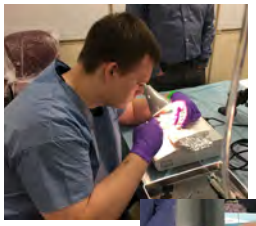
Hydrogel & Tool Prototype Production

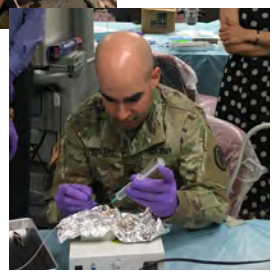
I. Benchtop Adhesion & Performance Tests → II. *in vivo* testing → III. End-User Feedback Workshop → Transition to Manufacturing

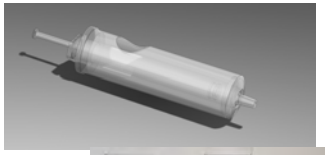






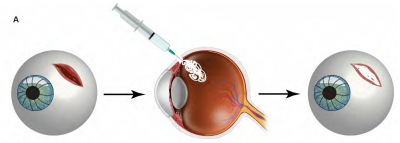


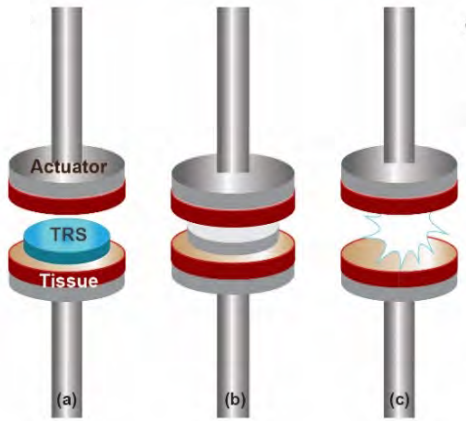


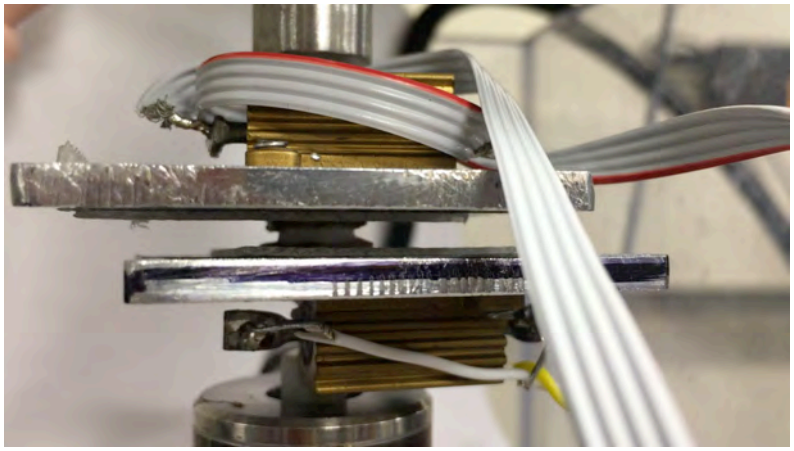


Bench Mechanical Adhesion Testing:

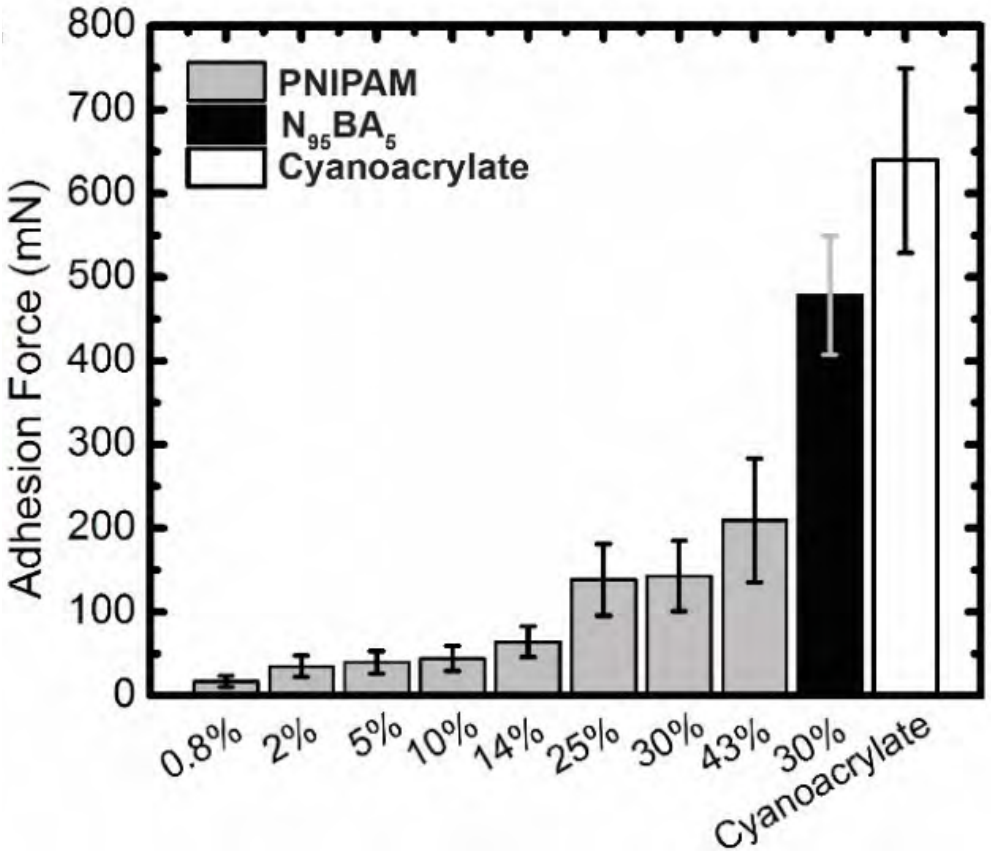
30% hydrated $N_{95}BA_5$ copolymer formulation provides adhesion strengths comparable to cyanoacrylate.







Mean maximum adhesion strength values recorded for different hydrogel sealant formulations.



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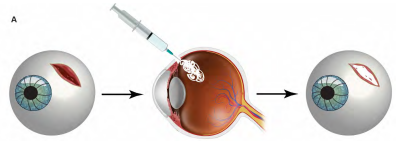
Bench IOP Sealant Testing.



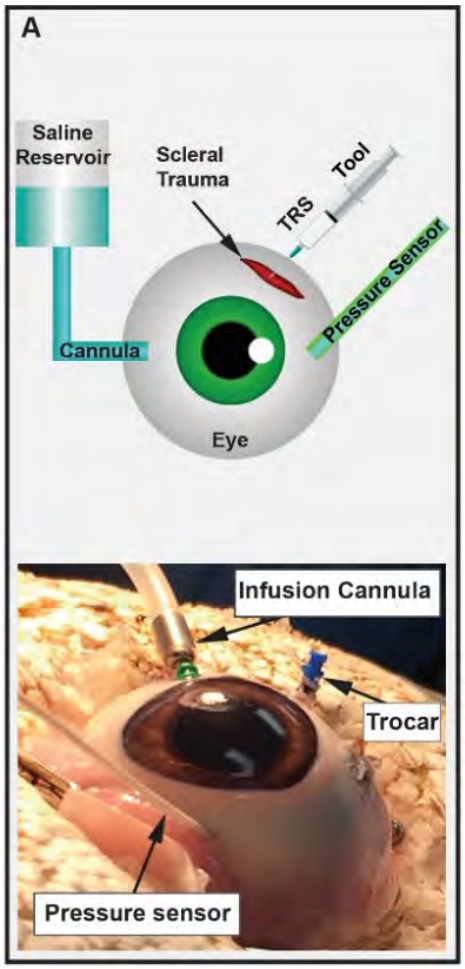
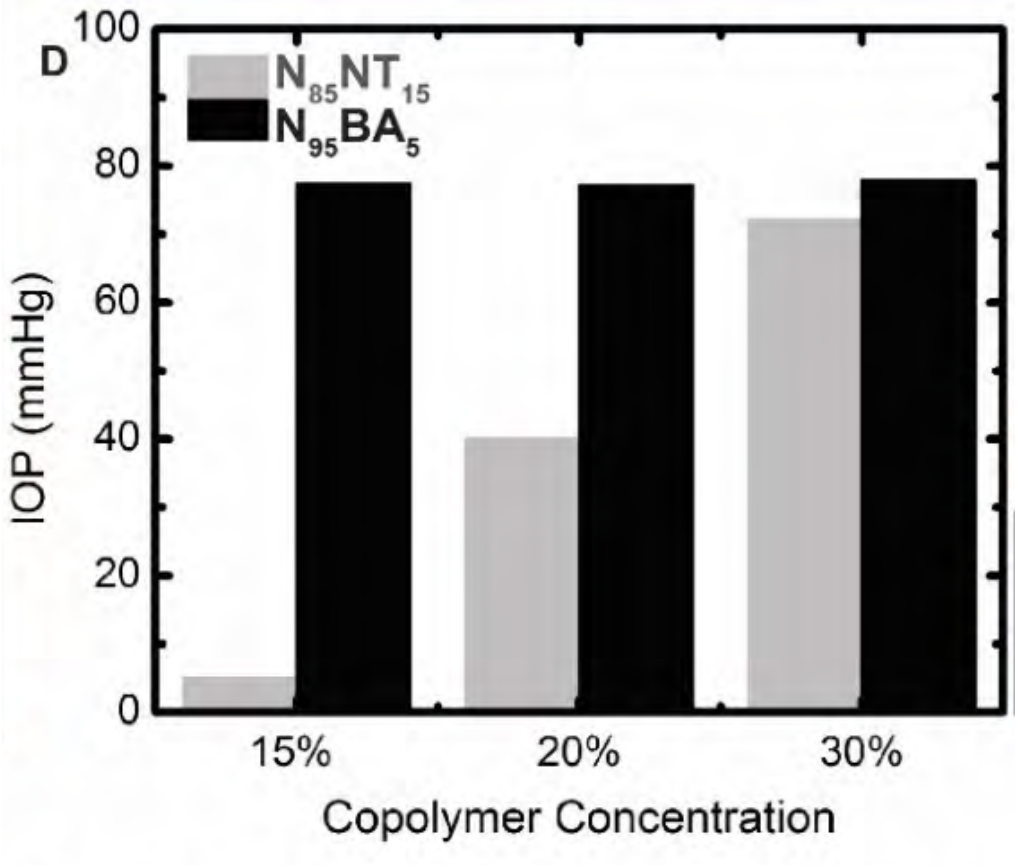
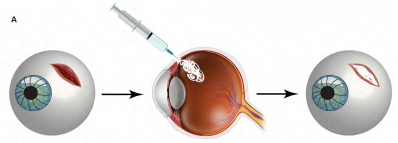


Fig 1. Schematic (top) and photograph (bottom) of bench top setup for assessing globe closure strength.

Fig 2. PNIPAM-Butylacrylate copolymer sealant can sustain IOP up to 80mm Hg before rupture/leakage.

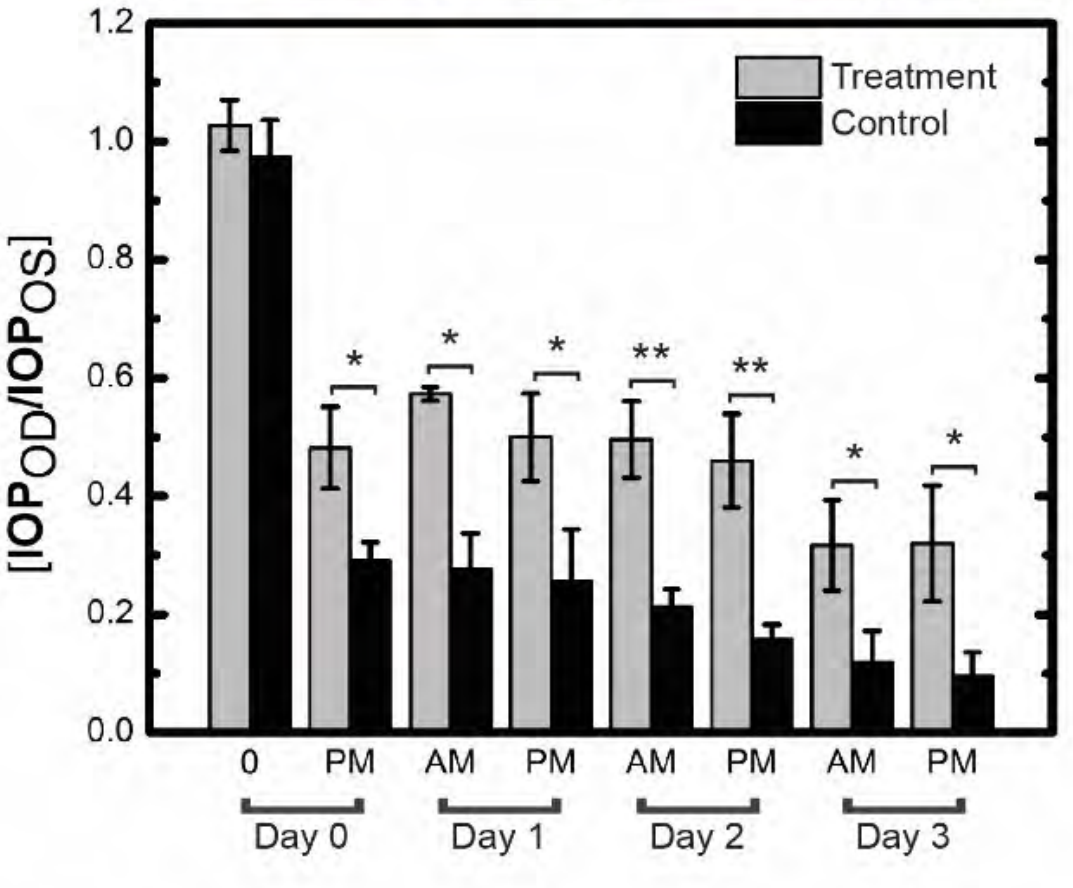


Deployment of hydrogel sealant in an in vivo model* of scleral perforation.



- In vivo assessment of sealant's ability to restore IOP and prevent leakage.
- Full thickness laceration (~5mm) all animals
- 2:1 → Hydrogel Sealant: No Intervention
- Followed 3-days → 30 days
- IOP, Histology
- Control: No intervention

Full-thickness lacerations treated with hydrogel sealant showed immediate and sustained IOP improvement over no intervention.



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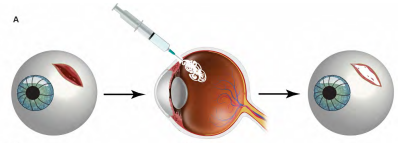
*These studies were conducted with review and approval from ACURO and USC IACUC.

4-week trans-scleral placement of the hydrogel sealant results in no evidence of chronic inflammation.



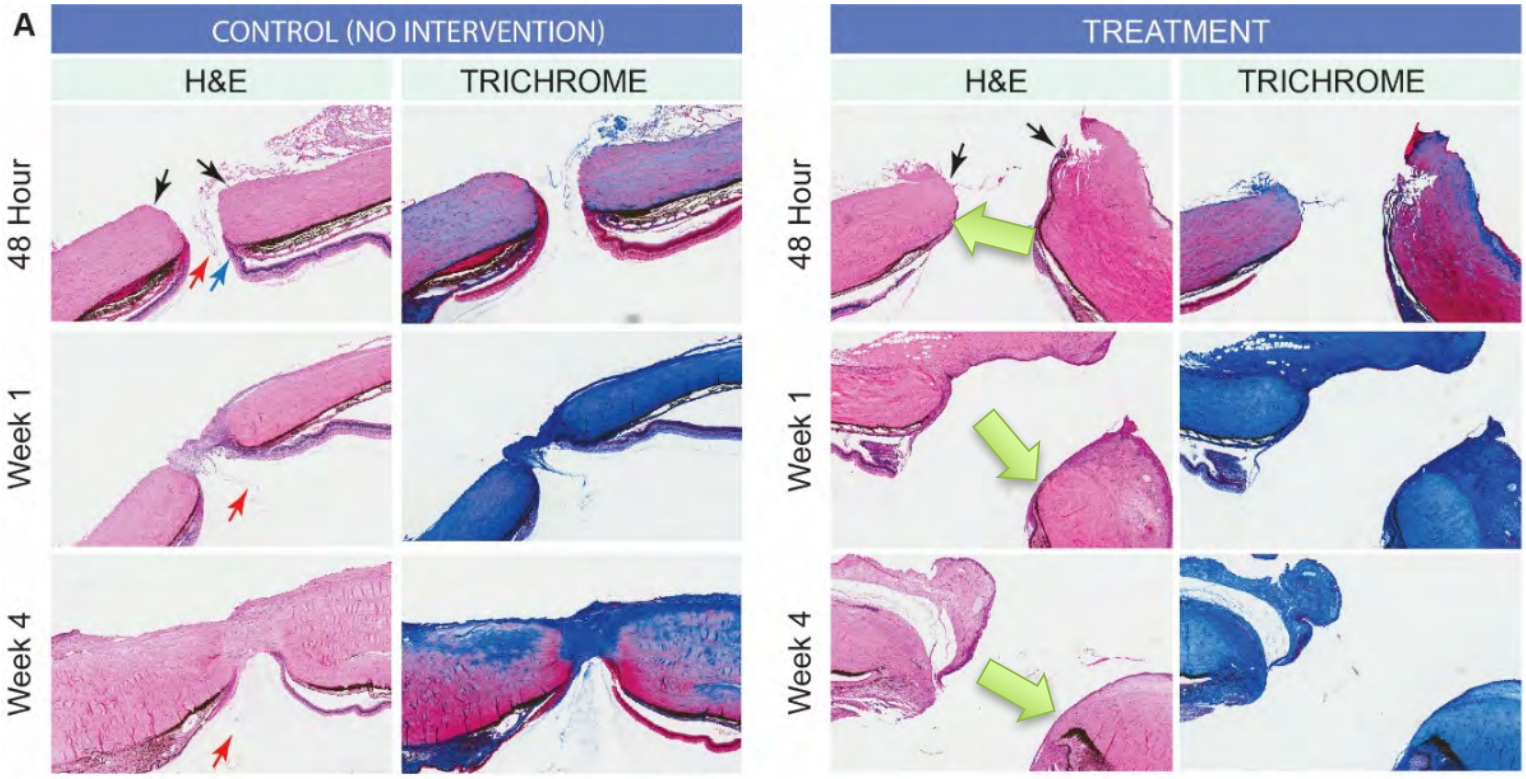
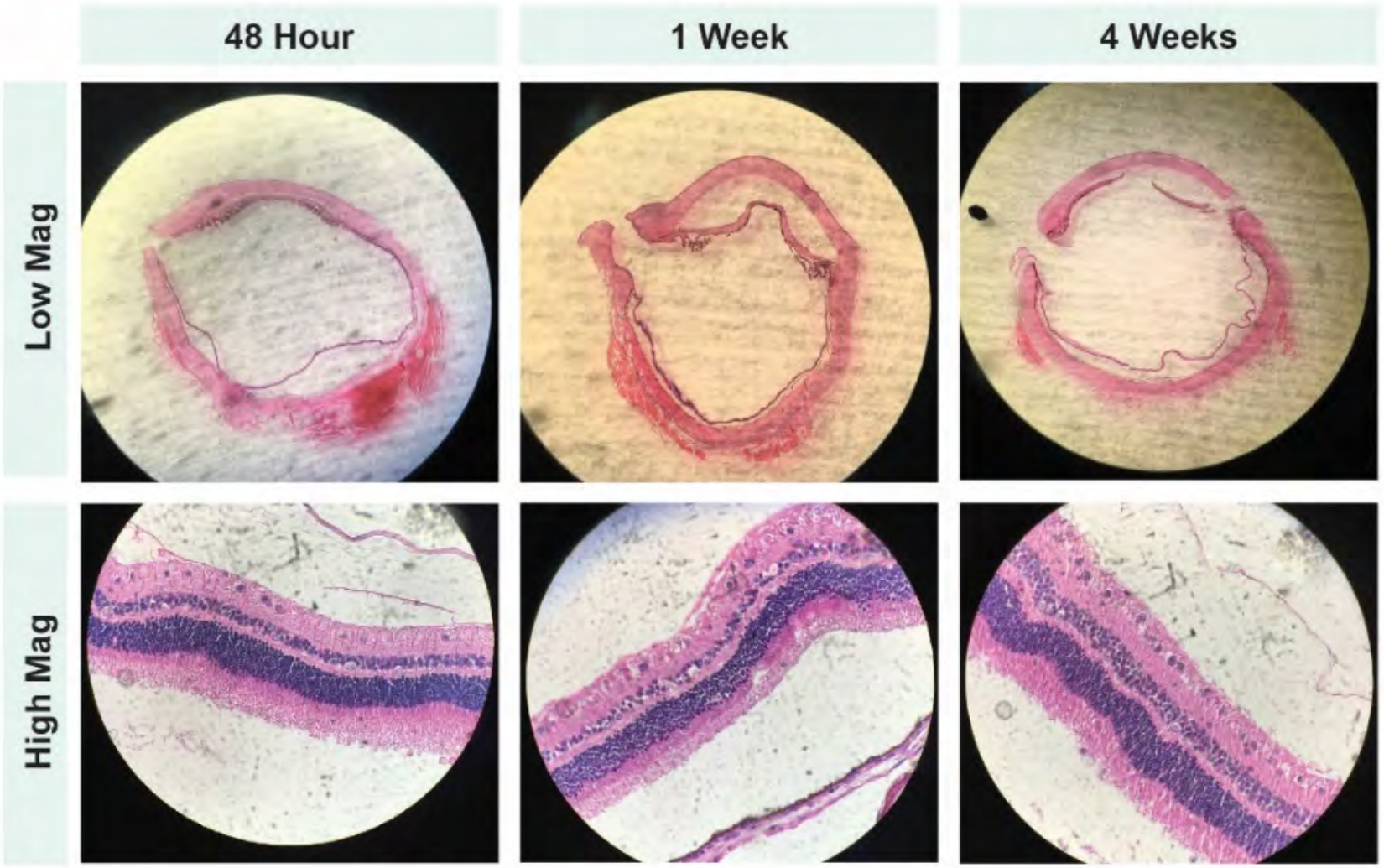
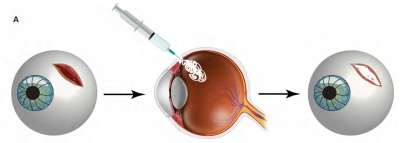


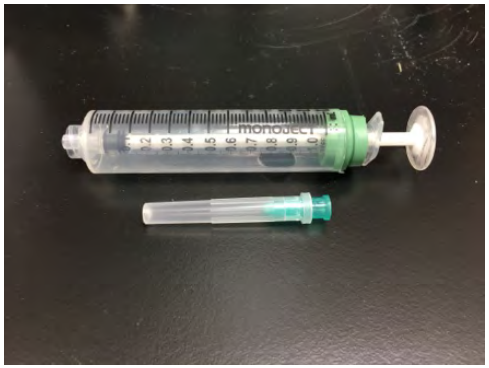
Fig 1. Trans-scleral placement of the hydrogel sealant results in no visible changes in retinal organization/structure out to 4-weeks.

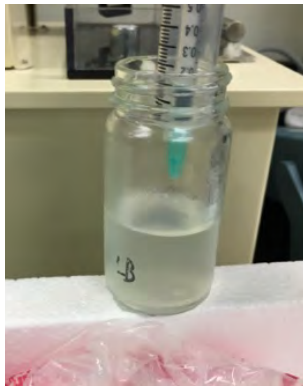


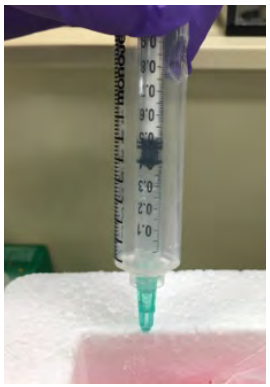
User Feedback from Military Ophthalmologists



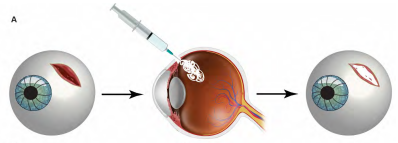
- Annual US Tri-Service Ocular Trauma Workshop
- Uniformed Services University/Walter Reed Medical Center.
- 140+ Registrants
- Military and civilian ophthalmologists, EMTs, medics & corpsmen.
- Produce ~150 injector tools each with 0.3-0.5cc sealant
- Packaged and shipped CA → DC







44 Participants in our User Feedback Workshop at the Annual Tri-Service

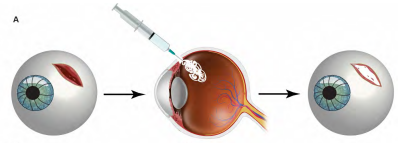


	Doctors	Medics/ Corpsmen	Other*
Day 1	7	0	0
Day 2	15	4	1
Day 3	15	0	2
	37	**4**	**3**

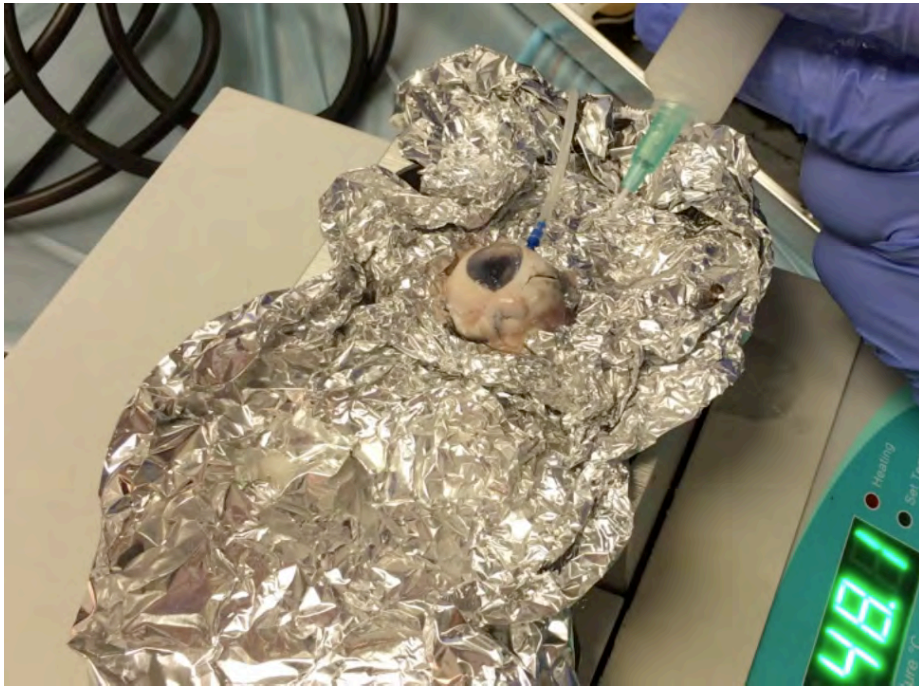
Age Breakout for Physicians

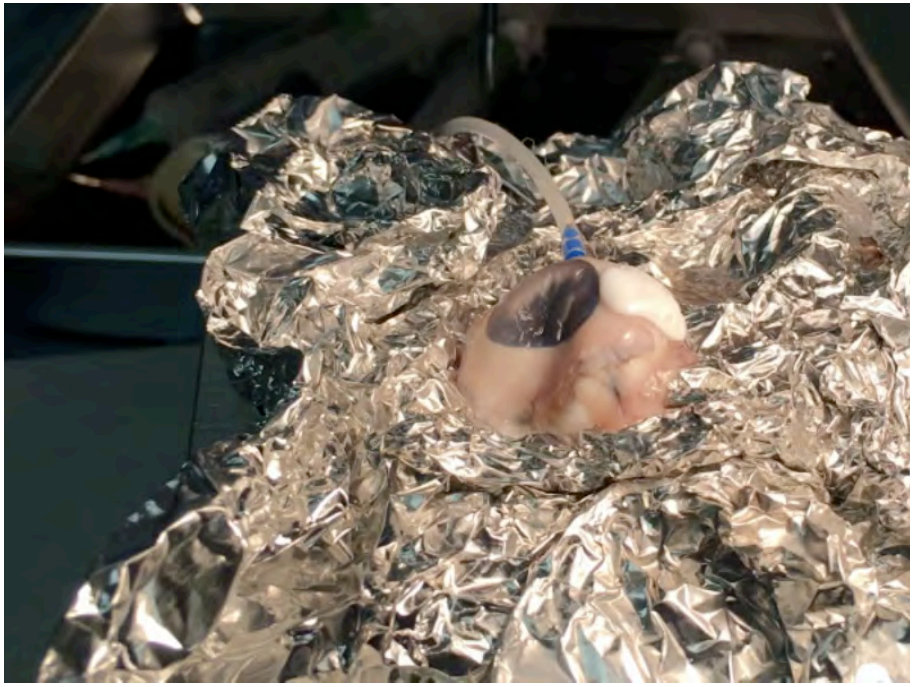
<30	31-40	41-50	51+
5	19	7	6

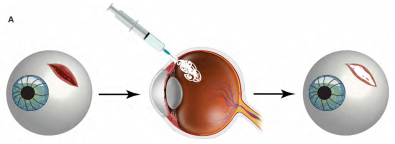
User Feedback Workshop

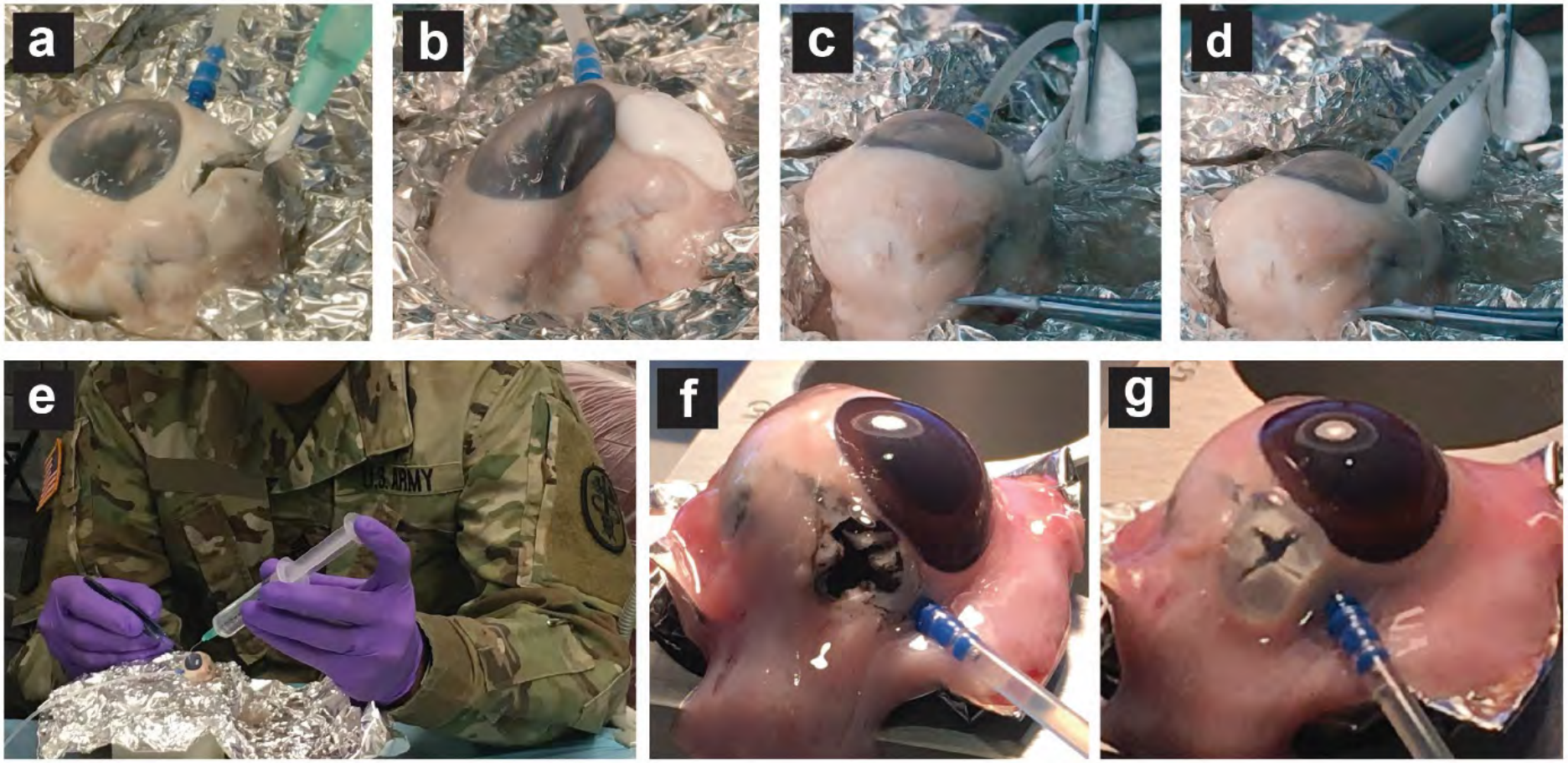


Hydrogel sealant placement by an ophthalmologist receiving minimal training (<15min) immediately prior to use.





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U.S. ARMY
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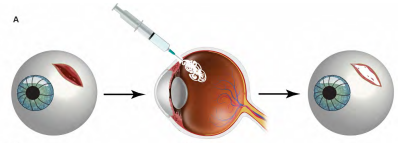
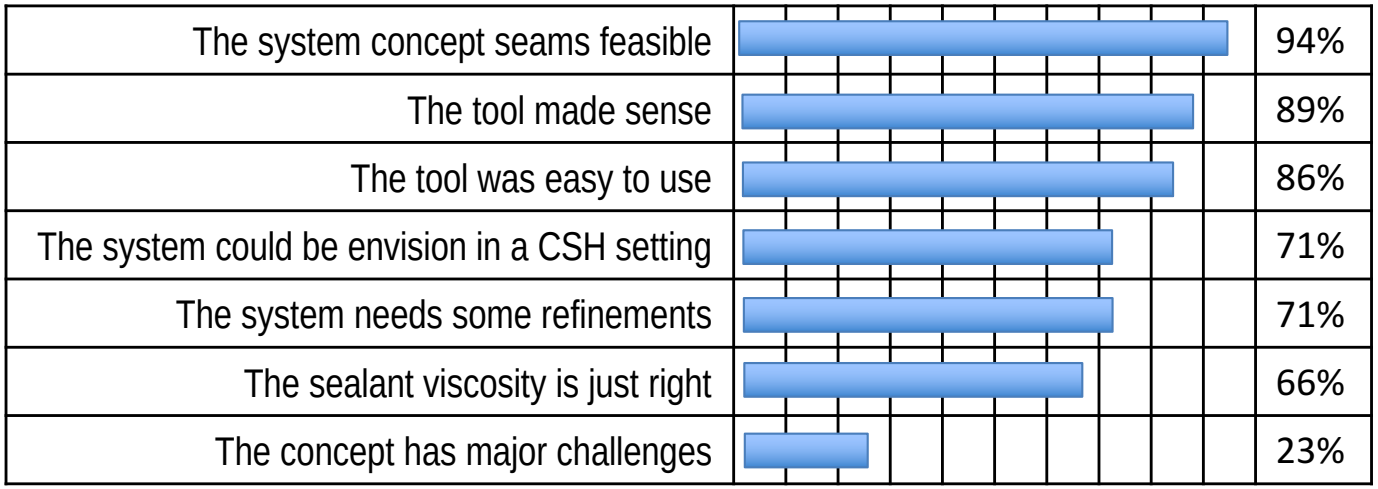
User Feedback



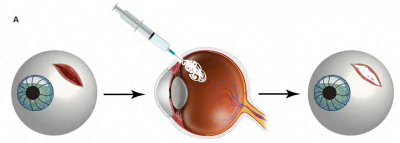
- **43% of testers closed globe on 1st attempt.**
- **100% of testers closed globe by 2nd attempt.**

Feedback from Respondents who Completed the Questionnaire



35 of 44 participants provided a complete multiple choice section.

Conclusions



- Reversibly deployable system developed.
- Thermoresponsive biocompatible polymer & disposable tool.
- Tested on the bench, in vivo and with military clinicians.
- Capable of occluding variety of globe ruptures quickly.
- Valuable user feedback was collected from military medical staff.
- Design being finalized → Assembling package for clinical testing.

Acknowledgements

USC Keck School of Medicine

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- Jose Cortez
- Fernando Gallardo
- Lina Flores

USC Dornsife College of Arts & Letters

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- Niki Bayat
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- Roby Menefee
- Andrew Bartinski PhD

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- Amal Nadel

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- Unrestricted Departmental Grant from **Research to Prevent Blindness**.

Thank you.

BIOMATERIALS

A reversible thermoresponsive sealant for temporary closure of ocular trauma

Niki Bayat,[1] Yi Zhang,[2,3] Paulo Falabella,[2,4] Roby Menefee,[5,6] John J. Whalen III,[2,3]* Mark S. Humayun,[2,3] Mark E. Thompson[1,7]

Open globe injuries are full-thickness injuries sustained to the eye wall (cornea or sclera), which cause immediate drops in intraocular pressure that may lead to retinal detachment and permanent vision loss if not treated rapidly after injury. The current standard of care for open globe injuries consists of suturing the margins closed, but the technique can be time-consuming, requires specialized training and equipment, and can lead to patient discomfort, abrasion, and infection from eye rubbing. We engineered an injectable, thermoresponsive sealant (TRS) and a custom tool to occlude open globe injuries. The smart hydrogel sealant consists of physically cross-linked *N*-isopropylacrylamide copolymerized with butylacrylate. At low temperatures, it can be injected as a liquid, and when raised to body temperature, a heat-induced gelation converts the hydrogel into a solidified occlusion. The sealant can be repositioned or removed without causing additional trauma via exposure to cold water. In vitro and ex vivo assessments of mechanical adhesion to eye tissue revealed maintenance of intraocular pressure that is five times greater than the physiological range with reversible seal strength comparable to cyanoacrylate (super glue). In vivo assessment in a rabbit model of ocular trauma demonstrated ease of use for TRS deployment, statistically significant improvement in wound sealing, and no evidence of neurotoxicity, retinal tissue degradation, or significant chronic inflammatory response after 30 days of exposure. Given the advantages of body heat–induced gelation, rapid reversible occlusion, and in vivo safety and efficacy, shape-adaptable TRSs have translational potential as smart wound sealants for temporary occlusion of surgical incisions or traumatic injuries.

INTRODUCTION

At least 2.5 million eye injuries occur in the United States each year, and open globe injuries account for 10% of these injuries (*1*, *2*). Open globe injuries can quickly escalate in complexity and yield poor visual outcomes if not managed carefully. Although incidence rates are relatively low, virtually all open globe injury patients see a reduction in visual acuity (VA), and the probability of VA loss increases with increasing time to intervention beyond 24 hours from the injury (*3*). In addition to affecting the quality of life of the patient, lifetime health care costs associated with visual impairment can approach $500,000, thus also having a major financial and societal impact (*4*).

Combat- and mass casualty–related ocular traumas are subsets of ocular injuries in which time to intervention is often delayed due to circumstances where patients are separated from medical services or triaged behind other casualties with more critical injuries. This delay in receiving treatment increases the risk for substantial visual impairment. In the U.S. campaigns in the Middle East, up to 13% of all casualties presented eye injuries, most attributed to improvised explosive devices (*5*), and between 20 and 40% of battlefield ocular injuries included penetrations to the sclera (*6*). A study of the Boston Marathon bombing determined that 13% of those injured required ophthalmology intervention (*7*). Military studies and clinical observations predict that treatments closest to the time of injury have the best outcomes (*8*). In these instances, developing a strategy to rapidly and temporarily close the globe without further trauma to the tissues is desirable.

Current treatment options for managing open globe injuries include sutures and adhesives. In general, these approaches require the use of microsurgical instrumentation accompanied by surgical microscopes to visualize tissue repair. Foreign body sensation resulting from abrasive material has been associated with eye rubbing, prolonged healing times, infection, and fibrosis (*9*). Novel bioadhesives like fibrin matrices have been used (*9*, *10*) but can carry considerable risks associated with assurance prevention of viral or prion contamination, in addition to challenges with glue deployment and ease of use. Cyanoacrylates (DERMABOND, TRUFILL, and DYMAX 222) exhibit some difficulties in dispensing and cannot be used to close globes with missing tissues (*11*). Acute inflammatory reactions in vascular tissue have been reported (*12*). There are currently no U.S. Food and Drug Administration–approved indications for using medical adhesives for closure of scleral penetrations.

Here, we propose a system designed to temporarily occlude open globe injuries. The system leverages the reversible, thermoresponsive properties of poly(*N*-isopropylacrylamide) (PNIPAM) to reversibly occlude injuries without causing additional trauma to surrounding tissues during placement or removal. PNIPAM is a smart biostable polymer investigated for a range of biomedical, drug screening, biotechnology, and medical diagnostics applications (*13*–*16*). Below about 32° to 33°C [lower critical solution temperature (LCST)], hydrophilic interactions of PNIPAM with water enable a translucent liquid state; above its LCST, it forms a partially dehydrated, soft-solid aggregate. Through copolymerization with other monomers, such as *N*-*tert*-butylacrylamide (NT) or butylacrylate (BA), we can tailor its thermoresponsive behavior and mechanical strength to create a hydrogel that shape-fills upon injection at a wound site, adapting to irregular margins and sealing traumatic injuries. The thermosensitive behavior allows the thermoresponsive sealant (TRS) to be easily removed by the application of cold water.

[1]The Mork Family Department of Chemical Engineering and Materials Science, University of Southern California, Los Angeles, CA 90089, USA. [2]USC Roski Eye Institute, Department of Ophthalmology, Keck School of Medicine, University of Southern California, Los Angeles, CA 90033, USA. [3]USC Institute for Biomedical Therapeutics, Keck School of Medicine, University of Southern California, Los Angeles, CA 90033, USA. [4]Department of Ophthalmology and Visual Sciences, Federal University of São Paulo, São Paulo 04023-062, Brazil. [5]Department of Biomedical Engineering, University of Southern California, 1042 Downey Way, Denney Research Center 140, Los Angeles, CA 90089, USA. [6]Department of Neuroscience, University of Southern California, 3641 Watt Way, HNB 120, Los Angeles, CA 90089, USA. [7]Department of Chemistry, University of Southern California, Los Angeles, CA 90089, USA.
*Corresponding author. Email: jjwhalen@med.usc.edu

We used this copolymer material to develop a reversible approach to temporarily occlude penetrating injuries to the posterior segment of the eye. In addition to tailoring the polymer chemistry, we developed a custom tool that controls the hydrogel temperature to enable effective deployment in the eye. Feasibility studies were conducted using ex vivo and in vivo models of ocular trauma in rabbits, and two user feedback workshops were held where military ophthalmologists tested the prototype systems for ease of use, general concept, and performance in an ex vivo porcine model of ruptured globe.

RESULTS

Engineered injectable TRS and mode of function

Our goal was to develop a biocompatible, tissue repair technology for temporary intervention at sites of scleral tissue damage or loss. The approach presented here involves injection of a purposefully nonbiodegradable liquid sealant capable of body heat–induced gelation to create a size-adaptable solidified occlusion, which can both restore intraocular pressure (IOP) and be easily removed within a few days for subsequent treatment without concomitant tissue damage. This technology would afford the patient a larger window of time to complete surgical intervention without requiring specialty equipment such as surgical microscopes for implementation. The system consists of two components: (i) a thermoresponsive hydrogel that, by its reversible transition from liquid to solid, conforms to wound shape and aggregates to mechanically seal scleral penetrations and (ii) a custom deployment tool to contain the hydrogel at a controlled temperature and to inject it at the targeted site (Fig. 1A).

Optimizing rheological properties of shape-persistent, moldable TRS

The physically cross-linked hydrogel with thermoresponsive behavior was designed from customized copolymer and water. The reversible transition from water-soluble coils to hydrophobic globules at the LCST changes the physical properties of the gel (Fig. 1B). Copolymerization of NIPAM with hydrophobic NT and BA not only decreases the LCST of PNIPAM but also improves the polymer's mechanical properties (*17*–*20*). By altering the compositional ratios of these copolymers, a range of formulations were synthesized via free radical polymerization and then characterized to optimize molecular weight, LCST, aqueous solution concentration, and viscoelastic properties (fig. S1). Previous studies have used both comonomers to create cell culture supports and other thermoresponsive polymers but not with synthetic uniformity or properties that met our needs (*18*, *20*). As a result, we followed a different synthetic scheme from those found in the literature and performed the necessary measurement of LCST and molecular weight for all formulations: homopolymers of NIPAM, copolymer of NIPAM with NT, and copolymer of NIPAM with BA, each using NIPAM as the main formulation component (Fig. 1C). Composition ratios for each sample were verified by ^{1}H nuclear magnetic resonance (NMR) (fig. S2).

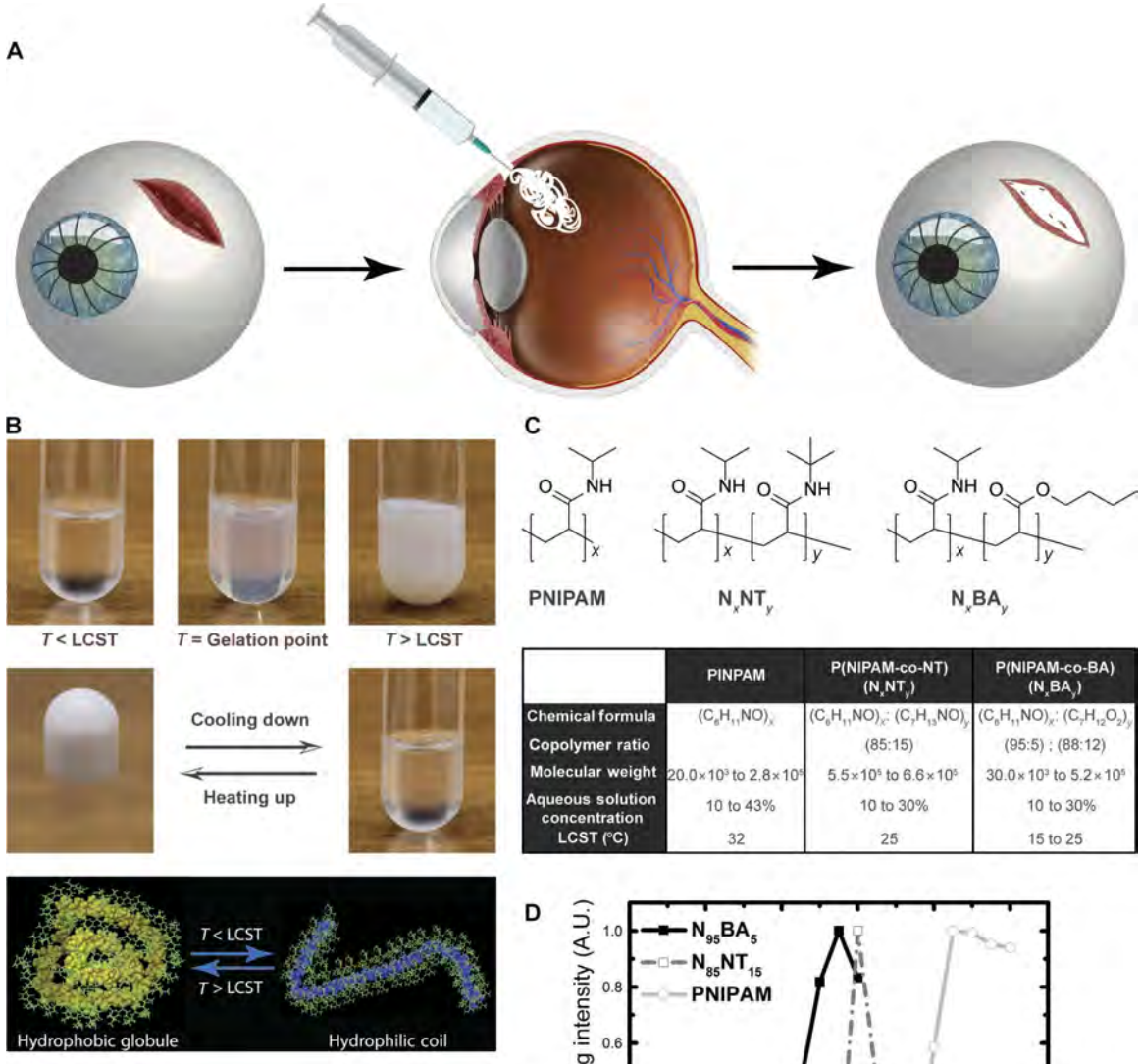
We deliberately engineered the LCST of the polymers studied here to fall below that of PNIPAM to achieve a transition temperature for the hydrogel that is well below the eye's physiological temperature. The contributions of the NT and BA monomers to the phase transition were examined through scattering intensity measurements of aqueous PNIPAM, poly(NIPAM-*co*-NT) ($N_{85}NT_{15}$), and poly(NIPAM-*co*-BA) ($N_{95}BA_5$) solutions over temperature ranges that included their phase transition temperature (fig. S3). Higher scattering intensity was observed at higher temperatures (above phase transition temperature), which was attributed to transformation from a more soluble coil conformation below the LCST to a largely insoluble compact conformation. For instance, the scattering intensity values of $N_{95}BA_5$ exhibited sharp increases around 16°C, indicative of a gelation point. Comparison of temperature-dependent scattering intensity distributions of three different hydrogels confirmed a PNIPAM gelation point around 32°C. By inclusion of only 5% BA or 15% NT, the gelation points shifted to 16° and 22°C, respectively (Fig. 1D). We successfully engineered smart hydrogels, $N_{85}NT_{15}$ and $N_{95}BA_5$, with appropriate transition temperatures for human eye application.

The viscoelastic properties of the hydrogels were determined by rheological analysis. Conventional elastic responses at LCST were demonstrated for both $N_{95}BA_5$ and $N_{85}NT_{15}$ samples (*21*). The loss (G'') and storage (G') moduli—representations of the viscous and elastic behavior, respectively—were measured by strain amplitude sweeps across a range of temperatures (figs. S4 and S5). Without a high-enough storage modulus above LCST, the hydrogel would not be sufficiently elastic to resist intraocular pressures while maintaining an effective occlusion. Without an optimized loss modulus below LCST, the hydrogel might be too runny or thick, making it difficult to apply. The storage modulus for $N_{95}BA_5$ rapidly decreases above the critical strain region (1%), indicating gel collapse to a quasi-liquid state (Fig. 2A). For different temperatures, the respective G'' (6°, 24°, and 32°C) and G' (24° and 32°C) values of $N_{95}BA_5$ and $N_{85}NT_{15}$ were measured as a function of angular frequency at fixed strain (0.1%) (Fig. 2, B and C). We observed that hydrogel dynamic moduli depend on the temperature and that both samples reached their maximum viscosity and elasticity at 32°C. Although the mechanical strength of both hydrogels showed the same trend, $N_{95}BA_5$ generated stronger polymeric networks. Replacement of NT comonomer with BA caused the G'' and G' values to increase by a factor of up to 30.

Comparison of the viscoelastic profile of 10% (w/w) aqueous solutions of two different copolymers at eye temperature (32°C) differentiated their aggregation behavior. G' dominates over G'' for $N_{95}BA_5$, resulting in a quasi-solid state ($\tan\delta \equiv G''/G' \approx 0.4$). Under the same condition, viscosity remained relatively greater than the elasticity for $N_{85}NT_{15}$, indicating a quasi-liquid state ($\tan\delta \equiv G''/G' \approx 2.3$) (Fig. 2, D and E). $N_{95}BA_5$ was selected for further characterization, owing to its mechanical strength and desirable phase transition temperature.

Viscosity measurements of $N_{95}BA_5$ provided a better understanding of the hydrogel's conformational change (Fig. 2F). Below the gelation point, complex viscosity, η^*, was independent of temperature and constant. At and above the phase transition region, the η^* rose sharply and approached a constant value. This change resulted from copolymer dehydration, compact globule formation, and resulting polymeric networks between macromolecule chains.

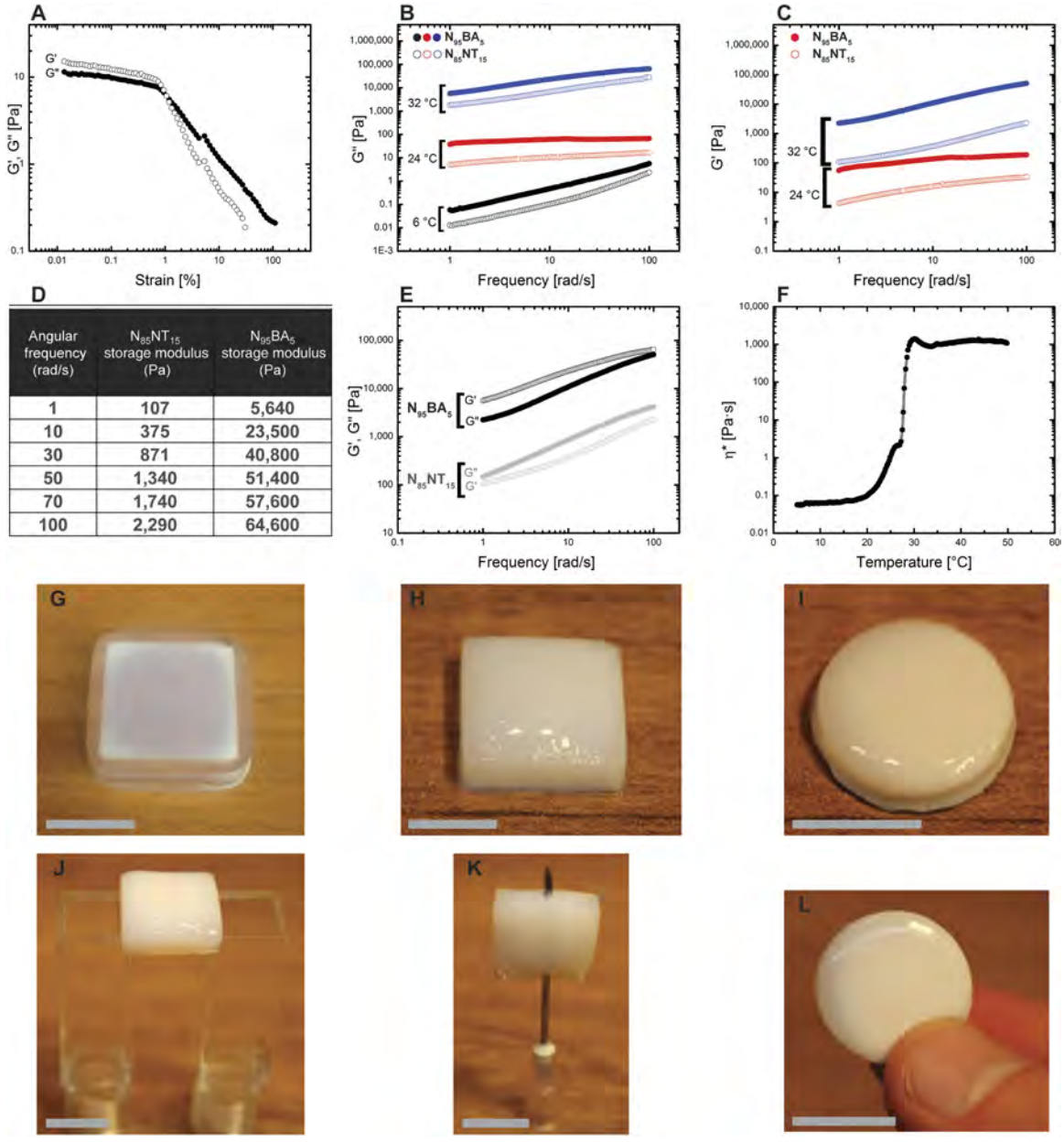
The complex viscosity profiles of $N_{95}BA_5$ solutions [5, 20, and 30% (w/w)] were then evaluated to compare their concentration-dependent strength. Earlier gelation onset accompanied higher concentration, indicating that more concentrated polymer solutions form gels at respectively lower temperatures. The η^* values also confirmed that higher copolymer concentrations can improve the mechanical strength of noncovalent hydrogels. Among the three samples studied, 30% (w/w) had the greatest complex viscosity value of about 10,000 centipoise (cP) (10 Pa · s), suggesting a strong yet injectable thermoresponsive hydrogel (fig. S6). For comparison, the viscosities of honey and ketchup, two easily applied yet malleable substances, are about 3000 and 50,000 cP, respectively. The 30% (w/w) $N_{95}BA_5$ was chosen for having the most useful viscoelastic properties of the hydrogels prepared herein (Fig. 1C).



	PINPAM	P(NIPAM-co-NT) (N_xNT_y)	P(NIPAM-co-BA) (N_xBA_y)
Chemical formula	$(C_6H_{11}NO)_x$	$(C_6H_{11}NO)_x$: $(C_7H_{13}NO)_y$	$(C_6H_{11}NO)_x$: $(C_7H_{12}O_2)_y$
Copolymer ratio		(85:15)	(95:5) : (88:12)
Molecular weight	20.0×10^3 to 2.8×10^5	5.5×10^5 to 6.6×10^5	30.0×10^3 to 5.2×10^5
Aqueous solution concentration	10 to 43%	10 to 30%	10 to 30%
LCST (°C)	32	25	15 to 25

Fig. 1. Design of a thermoresponsive hydrogel to seal scleral perforation. (**A**) Schematic depicting implementation of a temperature-mediated adhesive hydrogel that adapts to wound shape and a tool for its deployment at a perforation site in the sclera. (**B**) Images of the changes in physical properties as the sealing hydrogel transitions from hydrophilic coils to hydrophobic globules at its lower critical solution temperature (LCST). (**C**) Molecular structures of formulations of poly(*N*-isopropylacrylamide) (PNIPAM) with butylacrylate (BA) or *N*-*tert*-butylacrylamide (NT) and a table of resulting alterations of molecular properties and LCST values. (**D**) Normalized scattering intensity as a function of temperature for PNIPAM, poly(NIPAM-*co*-NT) ($N_{85}NT_{15}$), and poly(NIPAM-*co*-BA) ($N_{95}BA_5$). A.U., arbitrary units.

The stiffness and resilience of the 30% (w/w) $N_{95}BA_5$ were evaluated using standard tensile and compression tests and by casting it into shapes. Compressive stress-strain curves illustrated a positive correlation between TRS concentration and compressive moduli. The results revealed that the mechanical properties of the developed hydrogel can be readily tuned by changing the hydrogel concentration, with compressive moduli modified from less than 15 to ≈55 kPa when doubling the TRS concentration from 15 to 30% (fig. S7). The elastic modulus of $N_{95}BA_5$ was characterized as a function of the concentration (20, 25, and 30%). TRS tensile stress-strain curves followed a similar trend, with increasing copolymer concentration resulting in higher Young's modulus. The 30% TRS hydrogels were found to have elastic moduli of 117 kPa, representing an improved stretching capacity compared to 60 kPa (25%) and 45 kPa (20%) (fig. S8). The resulting molded objects (Fig. 2, G to I) were resilient and sufficiently cohesive at body temperature to be suspended horizontally (Fig. 2J), vertically (Fig. 2K), and by hand (Fig. 2L). The noncovalent properties of the hydrogel also allowed it to self-heal when slightly deformed. The hydrogel satisfied critical requirements for application as an ocular sealant: moldability, persistence in form, and sufficient toughness to withstand intraocular pressure.



Angular frequency (rad/s)	$N_{85}NT_{15}$ storage modulus (Pa)	$N_{95}BA_5$ storage modulus (Pa)
1	107	5,640
10	375	23,500
30	871	40,800
50	1,340	51,400
70	1,740	57,600
100	2,290	64,600

Fig. 2. Rheological characterization and hydrogel differentiation. (**A**) Storage and loss moduli (*G′* and *G″*, respectively) over strain for $N_{95}BA_5$. (**B** and **C**) *G″* (B) and *G′* (C) modulus representations of viscoelastic behavior for co-BA and co-NT as a function of angular frequency at fixed strain. (**D**) Table of storage moduli for co-NT and co-BA compositions at different angular frequencies. (**E**) Storage and loss moduli (*G′* and *G″*, respectively) over frequency for co-NT and co-BA compositions. (**F**) Measurement of complex viscosity for $N_{95}BA_5$ according to temperature. (**G** to **I**) Images of square and round gel molds formed by heating the hydrogel solutions to 32°C. Scale bars, 1 cm. (**J** to **L**) Images of solid $N_{95}BA_5$ hydrogel demonstrating horizontal resilience (J), vertical resilience (K), and strength of form on contact (L). Scale bars, 1 cm.

Ex vivo and in vitro testing of $N_{95}BA_5$ for ocular trauma

Sealant efficacy of the hydrogel was first assessed in an ex vivo cadaveric porcine eye model of ocular trauma. Cyanoacrylate was selected over fibrin-, albumin-, and polyethylene glycol–based adhesives as a positive control because of its well-documented superiority in maintaining IOP and uniaxial adhesion strength (*22*, *23*). The hydrogel was applied to the lacerated eye model after an incision procedure (Fig. 3A). The hydrogel was injected into the posterior chamber of the eye through the perforation, and the injection tool was slowly retracted while continuously deploying the hydrogel, leaving a sealant trail through the wound. At the exterior surface of the sclera, additional hydrogel was deposited, creating a gel rivet-like cap. The caps were left to settle for several seconds, allowing them to increase in temperature and dehydrate before being cut away or smoothed flat (Fig. 3, B and C). IOP was then controllably raised by infusion with warm saline from the cannula inserted in the posterior chamber.

By comparing the IOP values established at eye temperature (32°C) by 15, 20, and 30% (w/w) aqueous solutions of $N_{95}BA_5$ and $N_{85}NT_{15}$, $N_{95}BA_5$ was determined to be the superior hydrogel, maintaining ocular pressures above 70 mmHg for all concentrations. Under the same conditions, IOP values for 15 and 20% $N_{85}NT_{15}$ were less effective,

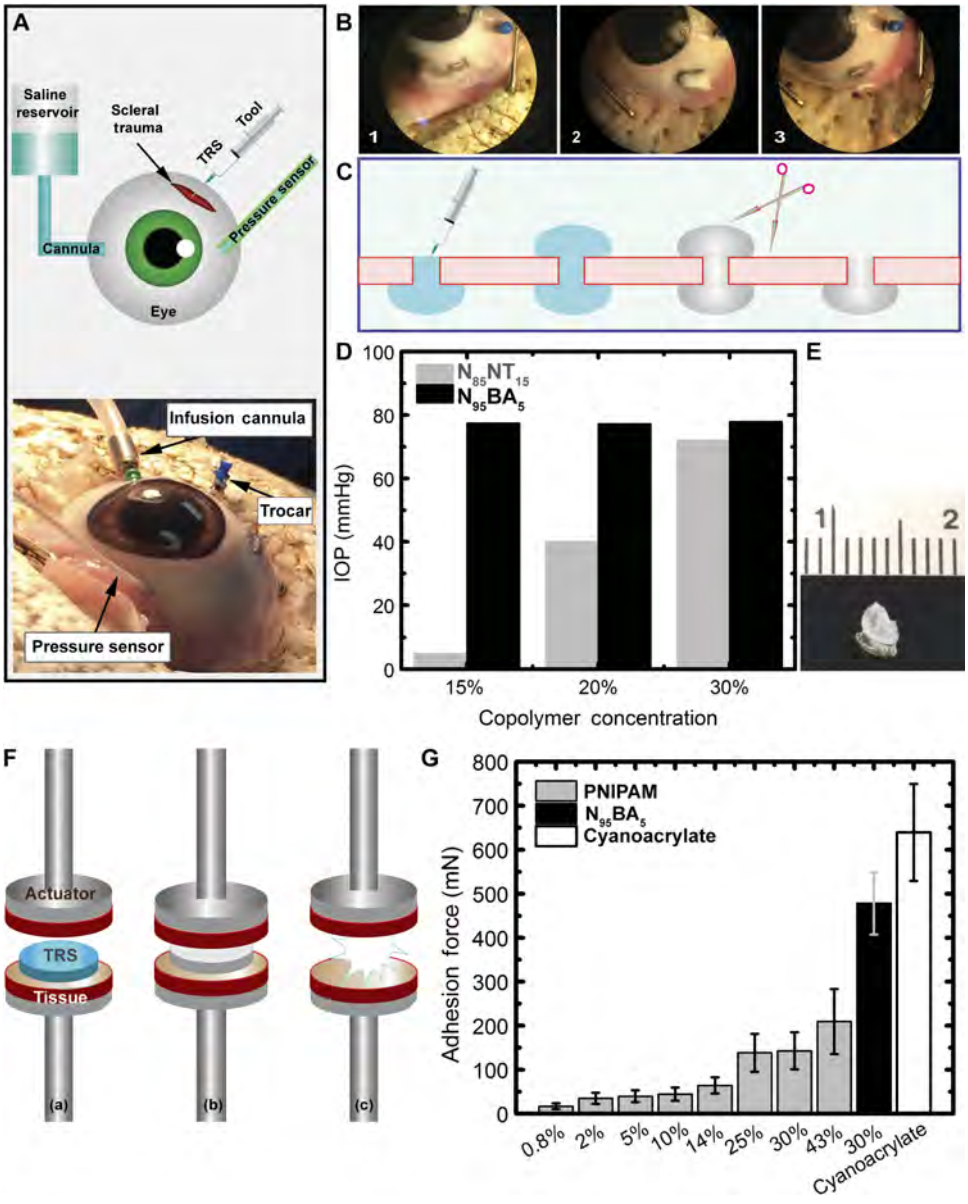


Fig. 3. Preliminary ex vivo and in vitro hydrogel evaluation. (**A**) Schematic (top) and image (bottom) depicting ex vivo procedures carried out in a pressure-controlled explanted cadaveric pig eye. (**B** and **C**) Images (B) and schematic depiction (C) of hydrogel injection through scleral perforation by deployment of a sealant trail through the wound, leaving rivet-like caps subsequently removed to leave the occlusion flush with the scleral surface. (**D**) Comparison of maintained intraocular pressures across a concentration spectrum for $N_{95}BA_5$ and $N_{85}NT_{15}$ hydrogels (n = 3 per group). (**E**) Image of a solid plug removed from a test eye. (**F**) Schematic depicting tissue adhesion tests comparing PNIPAM, $N_{95}BA_5$, and cyanoacrylate adhesion strength to scleral tissue ex vivo. (**G**) Adhesion force of different concentrations of $N_{95}BA_5$, PNIPAM, and cyanoacrylate to scleral tissue; columns show 478 ± 71 and 639 ± 110 for $N_{95}BA_5$ and cyanoacrylate, respectively (n = 3 per group).

containing pressures of 5 and 40 mmHg, respectively (Fig. 3D). Although high concentrations (30%) of both $N_{95}BA_5$ and $N_{85}NT_{15}$ hydrogels were capable of maintaining pressures of up to 72 mmHg, leakage was observed for $N_{85}NT_{15}$, indicating a quasi-sealed state. Even so, the formulations identified as effective could withstand IOPs of up to 77 mmHg without leakage, an environment approximately five times the physiological IOP range. Both the $N_{95}BA_5$ samples and a cyanoacrylate-sealed positive control held the maximum pressure (78 mmHg) of our experimental setup, demonstrating effective equivalence. In contrast, our negative control without a sealant held no pressure. Figure 3E shows a hydrogel plug removed from one of the rabbits at the end of the study.

Although we designed the hydrogel as an ideal mechanical sealant, in vitro uniaxial adhesion tests were performed to provide accurate comparison between the adhesion force of cyanoacrylate, the strongest ocular adhesive, and our TRS. The hydrogel or cyanoacrylate was sandwiched between two pieces of dissected scleral tissue that were fixated to the base and actuator arm of a pull tester (Fig. 3F). Apposed tissues were brought down and pressed together using 15g of pressure for 2 min. The actuator was then pulled until the two tissue samples detached. In addition to the 30% $N_{95}BA_5$, different aqueous concentrations of PNIPAM ranging from 0.8 to 43% (w/w) were chosen to assess the impact of concentration. Adhesion results of homopolymers suggested that an increase in the aqueous concentration of the hydrogel increased the adhesive strength between the hydrogel and scleral tissue. Adhesion forces between 336 and 560 mN were observed for the 30% (w/w) $N_{95}BA_5$ before adhesion failure at the tissue-hydrogel interface. Under the same uniaxial conditions, cyanoacrylate's adhesion force was slightly higher (Fig. 3G). Wetting, diffusion, and adsorption theories offer mechanical and molecular explanations for this behavior but were not explored in this study (*24*, *25*).

In situ gelation mechanism of TRS in the eye

Ex vivo application indicated that to occlude the incision properly, the hydrogel must change from transparent liquid to white solid (Fig. 3B). We used dynamic light scattering (DLS) techniques to obtain particle size in solution, gel and solution-to-gel transition states, and particle impact on gelation kinetic and mechanical properties. At 2°C, below LCST, we observed that 96% of the intensity corresponds to particles with a small hydrodynamic radius (3.6 nm), attributed to single polymer chains in the coil conformation (Fig. 4A). At the phase transition region, we observed two peaks with comparable intensities. One peak (236.2 nm) corresponded to $N_{95}BA_5$ molecules that were partially aggregated in the solution, and the other peak (4.5 nm) corresponded to lower-radius particles (Fig. 4B). Further temperature increase to 20°C caused 98% of the copolymer to form large aggregates (439 nm), a consequence of more favorable polymer-polymer

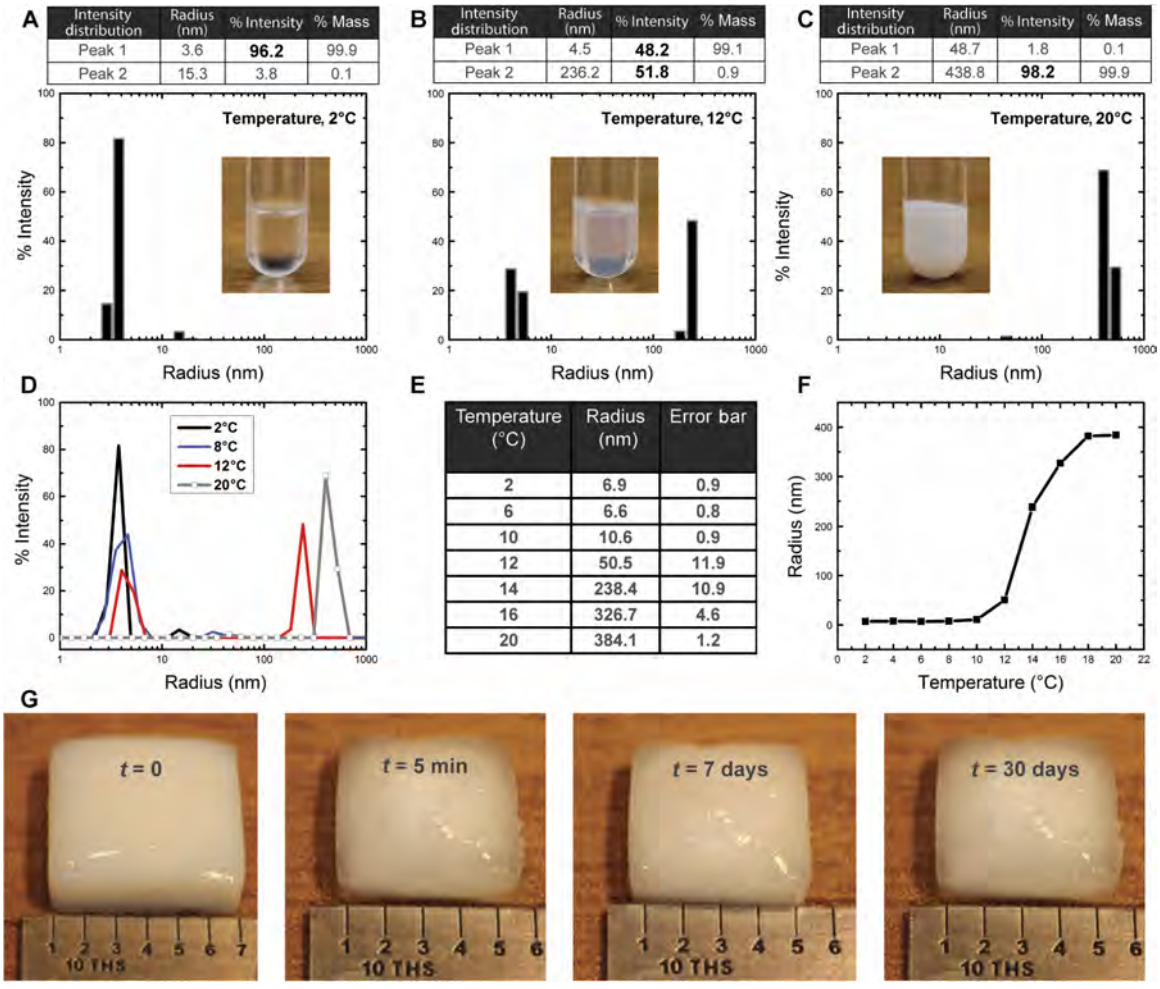


Fig. 4. Hydrogel particle size and gelation mechanism. (**A**) $N_{95}BA_5$ particle size as assessed using dynamic light scattering in solution below LCST (2°C), showing a 96% scattering intensity for small-radius particles. (**B**) Particle size in the phase transition region (12°C). A split was observed between particles with hydrodynamic radius of 4.5 and 236.2 nm. (**C**) Particle size toward the end of the phase transition region (18°C). Ninety-eight percent of scattering intensity was due to large-radius $N_{95}BA_5$ aggregates. (**D** to **F**) Hydrodynamic radius size of particles traced through the hydrogel transition to show higher aggregate populations at higher temperatures (n = 3 per temperature). (**G**) Gross images of molded hydrogel samples held at the expected eye temperature (32°C) for up to 30 days, showing slight volume decrease and good shape persistence and stability.

interactions (Fig. 4C). Amide groups facilitated those interactions by forming hydrogen bonds between polymer molecules, nesting themselves inside the globules.

Intensity distribution across a range of temperatures (2° to 20°C) allowed further characterization of the temperature-induced hydrophobic aggregation process (fig. S9). For brevity, the size distribution graphs of only four temperatures (2°, 8°, 12°, and 20°C) were included (Fig. 4D). With temperature increase, the first peak corresponding to smaller particles decreased in size. Simultaneously, hydrodynamic radius (R_H) and intensity of the high aggregation peak region increased with heat (Fig. 4D).

Analysis of R_H values for $N_{95}BA_5$ aggregates provided detailed understanding of the gel's temperature dependence. At temperatures below the LCST, R_H remained nearly constant (<10 nm) up to 10°C, but increasing the temperature above the 12°C gelation onset condition led to a rapid increase in the hydrodynamic radius and to sizes markedly larger than the PNIPAM aggregates (*26*). Greater aggregate size makes for better ocular sealant because larger particles diffuse less readily into the vitreous gel, as predicted by the Stokes-Einstein equation. The aggregation onset temperature was confirmed by the results of scattering intensity tests. DLS measurements could not be performed above 20°C because of the turbidity gain of the hydrogel solution (Fig. 4,E and F). In summary, the nanometric copolymer aggregates associate upon exposure to heat, becoming insoluble in aqueous solution as a consequence of more favorable polymer-polymer interactions.

As observed previously, heated hydrogel can form shape-persistent objects by in situ gelation. The thermo-induced volume change of these molded shapes was further characterized to investigate hydrogel stability in an environment similar to the eye. The swollen hydrogel underwent slight volume reduction after only 5 min of exposure to 32°C deionized water. Shape persistence and stability remained for 1 month in an aqueous environment above its LCST (Fig. 4G). These formed shapes were also maintained at 10°C, below LCST, resulting in complete structural collapse after a few hours, consistent with the thermoresponsive trends observed (fig. S10). Our hydrogel challenges the preconception that materials held together by noncovalent forces and mostly composed of water are weak.

Effective TRS deployment in an in vivo model of ocular trauma

Effective deployment of the hydrogel without premature transition required development of a controlled-environment injector tool to meet optimal use requirements derived from clinical end users (table S1). The preliminary design consisted of a 1-cm^3 chamber inside a larger 15-cm^3 thermal jacket (Fig. 5A, a) in an easily used form factor. On the basis of the preliminary design and volume of space available inside the thermal jacket, a series of endothermic chemical reactions of ammonium nitrate salt and water was performed and optimized to rapidly cool the hydrogel chamber to 0°C within 60 s of mixing and to maintain the hydrogel's temperature between 0° and 10°C for up to 10 min (Fig. 5A, b). A working prototype was fabricated from off-the-shelf parts, including a 1-cm^3 syringe nested inside a larger 15-cm^3 syringe and a soft removable loading port cap, which allowed ammonium nitrate to be loaded into the jacket and water to be injected in the jacket when ready for

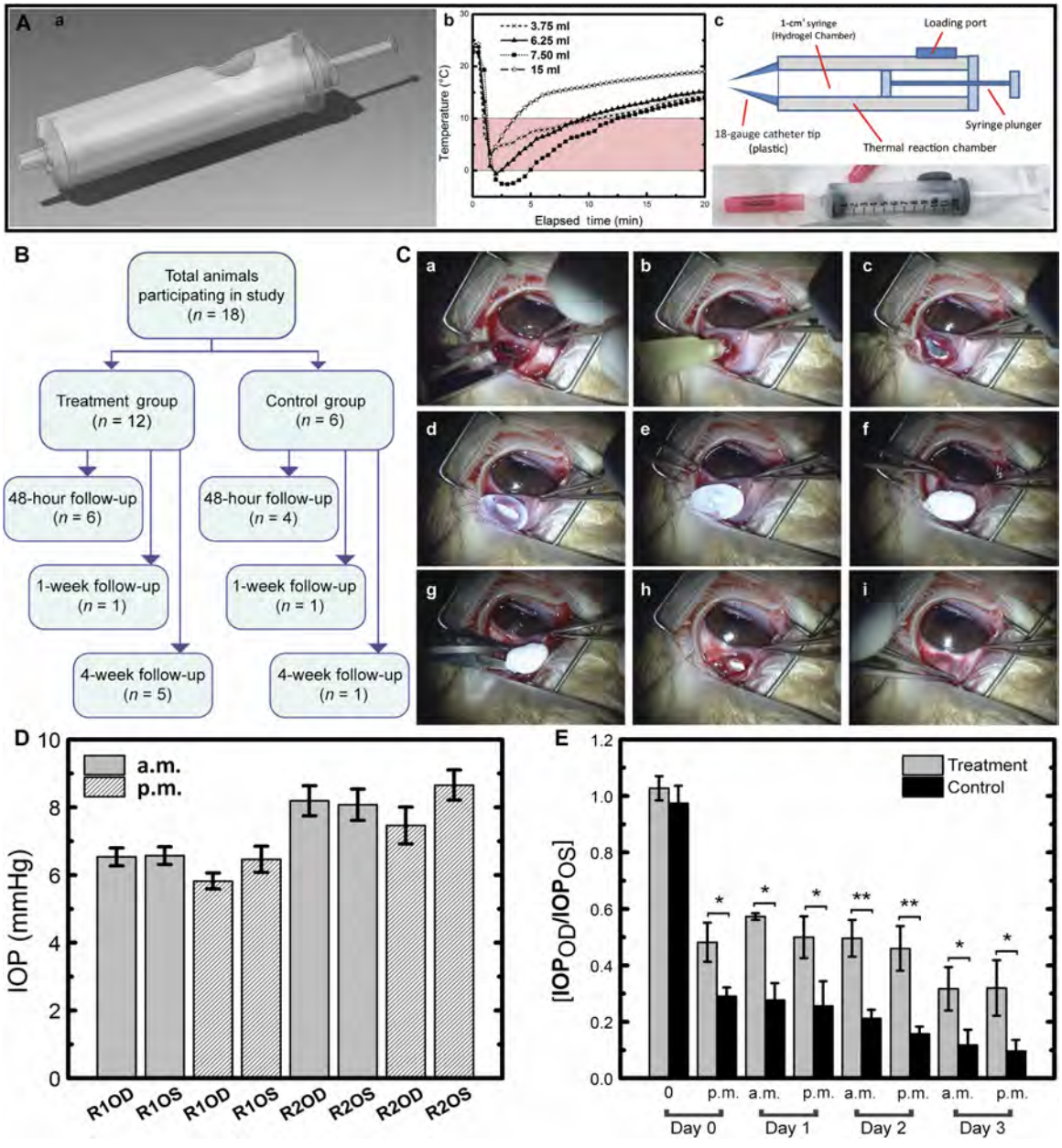


Fig. 5. Preliminary performance validation of the hydrogel in vivo in a rabbit model of scleral trauma. (**A**) Design diagrams (a and c) and validation (b) of a custom injection tool to effectively control hydrogel deployment and regulate its temperature. An image of the prototype injector is shown in the bottom right. (**B**) Two-arm study design to assess safety and efficacy of the hydrogel versus the current standard of care for posterior segment open globe injuries. (**C**) Images of the surgical procedure in rabbits. A 3-mm, full-thickness linear incision was created in the sclera about 3 mm radial from the limbus, followed by preparation and deployment of the hydrogel through the incision. (**D**) Representative baseline intraocular pressure (IOP) values showing no statistical difference between eyes of the same animal or any circadian-induced variations; columns show 6.5 ± 0.2, 6.5 ± 0.2, 5.8 ± 0.2, 6.4 ± 0.3, 8.1 ± 0.4, 8.0 ± 0.4, 7.4 ± 0.5, and 8.6 ± 0.4 mmHg (n = 28 per group). (**E**) Wald test comparison of mean IOP values of the treatment group versus no intervention, after procedure, showed a statistically significant improvement in mean IOP with sealant placed (*P < 0.05 and **P < 0.001).

use (Fig. 5A, c). Cooling characteristics were optimized by varying reactant concentrations and tracking temperature transients to achieve the desired deployment window (fig. S11). A polymer-based catheter cannula was also used on the injector tip for hydrogel application to reduce thermal conductivity from the eye, which could have induced temperature transition solidification in the tool's lumen during deployment.

An in vivo pilot validation study was performed in a rabbit model of ocular trauma to assess the ease of use, safety profile, and preliminary efficacy of the $N_{95}BA_5$ hydrogel. A 7-day in vivo follow-up study was performed to compare the performance of the hydrogel wound closure versus no intervention (control). A subset of the study animals were followed for an additional 3 weeks (4-week endpoint) to more carefully examine safety and potential inflammatory responses to hydrogel placement in the eye (Fig. 5B and table S2).

For each test run, a 3-mm full-thickness laceration was created in the sclera to simulate a penetrating injury (Fig. 5C, a). After trauma creation in a treatment group rabbit, the tool was inserted into the laceration (Fig. 5C, b) and the hydrogel was deployed (Fig. 5C, c). Once deployed, the hydrogel was then allowed to set for 5 min, enabling the dehydration transition from translucent (Fig. 5C, c and d) to an opaque white color (Fig. 5C, e). Expelled water formed visible droplets on the gel surface (Fig. 5C, f). The rivet cap formed on the ocular surface was then cut away to create a low-profile, flat head (Fig. 5C, g to i). The procedure can be viewed in movie S1. Ease of use of deployment was evaluated by comparing the number of successful hydrogel placements into the eye versus the number of hydrogel placement attempts.

Despite limited training with the system, the veterinary surgeon was able to successfully deploy the hydrogel on the first attempt in greater than 80% of the cases (table S2). During follow-up, trauma sites in the control group were difficult to locate. The conjunctival epithelium formed a fibrotic layer across the margins in an attempt to repair the breach. Although suggestive of a natural healing process, the eyes still exhibited hypotony with IOPs of ≤4 mmHg, likely attributable to a leaky, porous closure.

The scleral surface of all study eyes [right eye (OD)] was visually evaluated by an ophthalmologist at 24 hours, 48 hours, and 1 week to look for signs of scleritis or other vasculitis of surrounding tissues that might be indicative of adverse tissue responses to the hydrogel material (fig. S12). Despite some signs of acute inflammation in the first hours (less than 12 hours) after the original procedure, irritation rapidly subsided. By the 24-hour mark, treatment and control eyes were barely distinguishable. Evaluation at 48 hours after surgery revealed no hyperemia or inflammation at the treatment site, which persisted until study termination.

Hydrogel efficacy in situ was evidenced by IOP restoration to normal ranges and negative Seidel test, the standard clinical test for identifying ocular leakage/dehiscence. Mean baseline IOP values showed no statistically significant difference between the study eye (OD) and fellow eye (OS) of each animal (presurgical OD/OS between groups, $P = 0.35$), consistent with previous reports (Fig. 5D) (*27*). Immediately after incision creation, both treatment and control groups showed significant decreases (>80%) in mean-normalized IOP. As restoration of IOP relied on physiological production of aqueous humor, immediate improvement in IOP was not expected in both groups.

A noticeable IOP increase occurred about 12 to 24 hours after procedure in the treatment group, and statistically significant improvement in IOP continued over the 72 hours after placement of the hydrogel relative to the control (Fig. 5E and table S3). Normalized mean IOP measurements across all time points beyond 24 hours after procedure showed statistically significant improvement over control, with an even greater improvement noted beyond 48 hours ($P < 0.05$). Normalized to the mean normal clinical IOP (15.5 mmHg), the lower threshold for normal human IOP pressure (10 mmHg) becomes 65% (*28*), and unrepaired globes maintained a low IOP no greater than 30% of normal. In stark contrast, hydrogel closure consequently raised IOP to 90% of the minimum threshold for normal IOP and sustained IOPs almost twice the magnitude of no treatment.

$N_{95}BA_5$ biocompatibility up to 1 month after ocular implantation

Histological analysis was performed at 48 hours, 1 week, and 4 weeks after hydrogel implantation to investigate potential adverse reactions. Tissue cross sections with the laceration and hydrogel placement sites marked (Fig. 6A, black arrows) show the trauma site and hydrogel placement location. A comprehensive analysis of tissue reaction over intervention time course was drafted by a certified pathologist (table S4). Despite design intent for acute intervention, elongated exposure allowed inspection of chronic inflammatory reactions, usually observed at 3 to 4 weeks after an implantation (*29*).

At 48 hours, control group wound margins appeared clean with the presence of some acute inflammatory cells (Fig. 6A, top left). Partial evulsion of peripheral retina was noted at the margins (blue arrow) of the control group along with some inward epithelial migration (red arrow). At 1 week, the control eyes exhibited a typical acute inflammatory response, forming a porous fibrotic layer bridging the lesion margins that matured from a positive Seidel test (confirmed leakage) to a complete IOP-supportive barrier by week 4. At week 1, there was no evidence of inflammation or infection in scleral tissues, but epithelial migration into the posterior chamber was observed. Trichrome staining revealed the mature (week 4) barrier to be dense and primarily composed of collagen fibers, a natural healing response. Small distributions of inflammatory markers were found in the newly formed tissues, but there was no evidence of chronic inflammation.

Treatment group eyes exhibited similar clean laceration margins at 48 hours with some retinal tissue evulsion; however, the opposing margins were noticeably separated (Fig. 6A, right) as a result of the hydrogel occlusion. Acute inflammatory cells at wound margins and slight inward epithelial layer migration were consistent with acute traumatic injury. An immature encapsulation layer formed over the wound by week 1, with no evidence of lesion closure or epithelial bridging, providing a quantitative metric for the foreign body reaction (Fig. 6B). The layer had a moderate thickness (51.0 ± 15.7 μm) of 10 to 20 cell layers. At 4 weeks, the encapsulation matured into a compact (13.4 ± 4.9 μm) tissue layer lining the margins of the lesion. Trichrome staining confirmed this layer to be rich in collagen, indicating evidence of a mature and compact fistula with a minimally adverse tissue response and no evidence of chronic inflammation forming around the hydrogel plug. The sclera also created an immature encapsulation layer containing some inflammatory cells, which separated the scleral tissue from the implant. Despite small quantities of infiltrate observed in the scleral margins at week 1, there was no evidence of sustained or excessive infiltrate, giant cell formation, or other chronic inflammation indicators at week 4.

Treatment group retinas showed no evidence of detachment and showed no evidence of neurotoxicity after 4 weeks of exposure (Fig. 6C). Different approaches have been used previously to assess neurotoxicity, including neurophysiological electroretinogram recordings

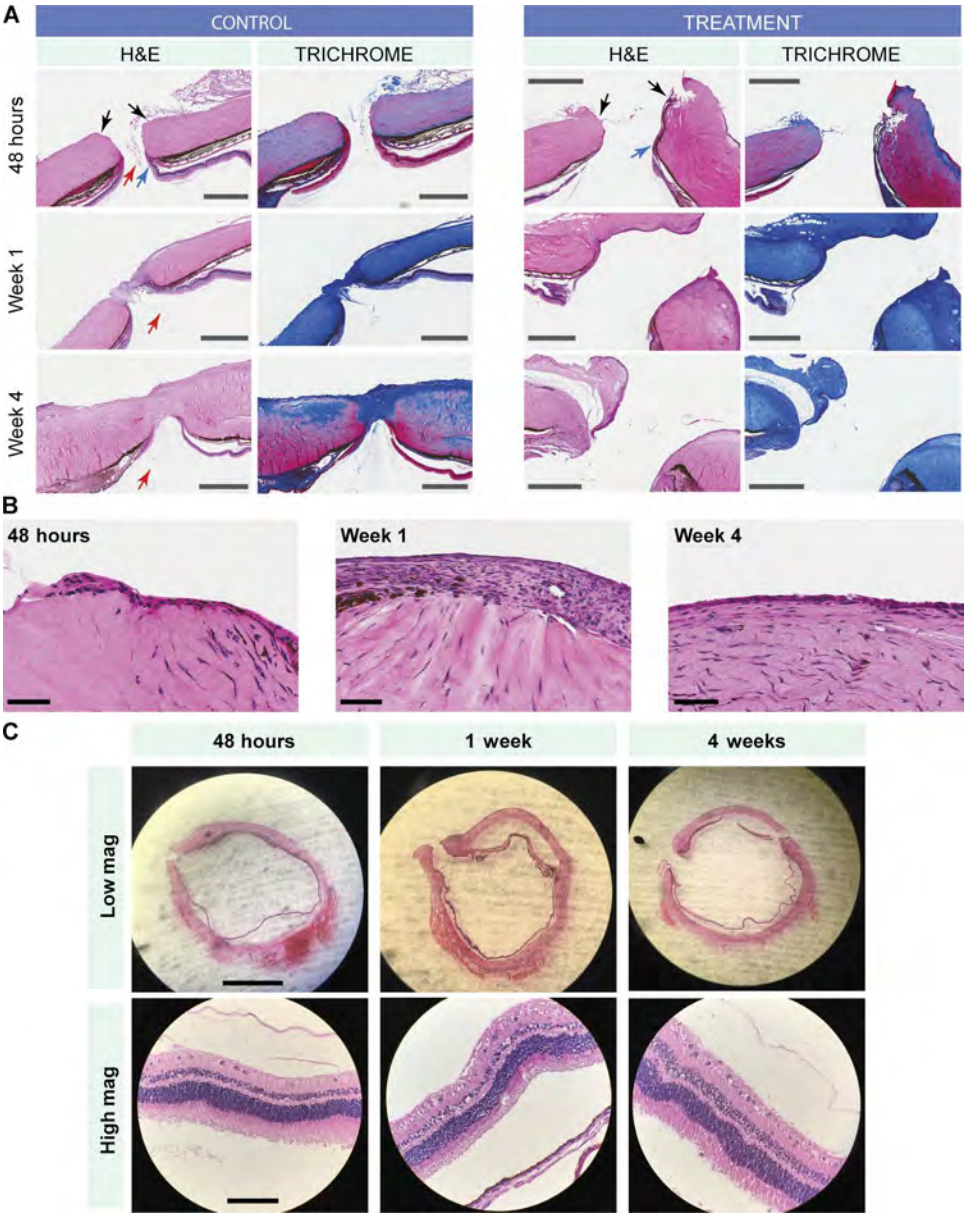


Fig. 6. $N_{95}BA_5$ biocompatibility beyond intended use frame. (**A**) Series of histological cross sections prepared for control (left pairs) and treatment (right pairs) in hematoxylin and eosin (H&E) and Masson's trichrome stain for each of the study endpoints (t = 48 hours, 1 week, and 4 week). Scale bars, 800 μm. (**B**) Increased magnification of one of the laceration margins for the treatment group showing evolution of the tissue-hydrogel interface from acute inflammatory infiltrate to a mature, compact fibrotic encapsulation layer at 4 weeks. Scale bars, 50 μm. (**C**) Gross visualization (top row) and high-magnification (bottom row) evaluation of treatment group retinas showing no evidence of trauma-induced retinal detachment or hydrogel-induced retinal neurotoxicity. Scale bars, 5 mm (top row) and 100 μm (bottom row).

and histological study of retinal structure (*30*). Treatment group physical retinal evaluation showed no signs of photoreceptor outer segment degeneration or other evidence of disorganization in the laminar tissue structure (Fig. 6C, bottom row). Organized ganglion cell layers, inner and outer plexiform layers, and nuclear layers were also present, along with retinal photoreceptor outer segments, all consistent with normally functioning retina. Any separation between the retina and choroidal layers was confirmed as artifacts of the histological slide preparation, which is a common observation (*31*). Even 30 days after implantation, the treatment group retinal tissues showed no signs of degradation or detachment.

User feedback workshop with military ophthalmologists and technicians

To validate the potential implementation of this technology in the clinic, two user feedback workshops were organized to allow military clinical personnel, who might see ocular trauma casualties, to use the TRS system in a benchtop model of ruptured globe injury (fig. S13). Two user feedback workshops that collectively captured end user feedback from 53 clinicians (ophthalmologists, physicians, medics/technicians, and researchers) with experience or career interests in managing ocular trauma in combat casualties were organized. The objective of these exercises was to assess the clinical relevance of the technology design and to capture any additional design refinements.

Each participant was given a brief tutorial and then asked to treat an enucleated pig eye with a full-thickness scleral laceration (0.5 to 2.0 cm in length). Each participant was given two attempts to close the globe. After sealant placement, warm saline was infused into the eye via a cannula to test integrity. Forty-three percent of the participants were able to successfully deploy the hydrogel at the site to effectively occlude the ruptured globe on the first attempt. All of the participants (100%) were able to successfully deploy the hydrogel at the site of the injury and close the globe in two attempts.

A questionnaire was administered to the participants with write-in (table S5) and multiple choice (table S6) questions. Of the responses collected, 69% of the respondents (n = 22) thought the idea of the reversible occlusion for open globes was a good idea (table S5), with 59% thinking it was great. Thirty-one percent found the system easy to use, with 25% noting that the system required minimal training. Perhaps most interesting, 94% of the responders (n = 30) said that they could envision the system being used in the field or in managing combat casualties.

DISCUSSION

PNIPAM and its copolymers have been extensively explored, but the combination of NIPAM and BA to target a biologically relevant transition temperature and mechanical properties pursues a unique trajectory.

Previous studies have imbued custom copolymers with the temperature sensitivity of PNIPAM, achieving pH-resistant graft copolymers, altered LCSTs, and temperature-dependent drug diffusion (*13*, *32*). Responsive properties of PNIPAM have also been applied to acylated polymer-bound parylene C for temperature-mediated tissue binding (*33*). With these dynamic modifications in mind, PNIPAM was an obvious foundation for an impermanent, biological sealant. To be used in the eye at 32°C, however, the LCST needed to be lowered.

Selection of the ideal copolymer conformation required examination of temperature-dependent aggregation behavior. Previous studies of PNIPAM copolymer behavior have used viscosity and DLS measurements to characterize particle size and shape (*34*). Our findings verified the concentration-dependent properties of hydrogel aggregation for this engineered conformation—higher concentration slightly lowered LCST—and reaffirmed non-Newtonian characteristics observed in studies of additive effects (*35*, *36*). The quasi-solid state of the 30% $N_{95}BA_5$ hydrogel, in conjunction with its lowered LCST, distinguished it as the ideal candidate. Potential further improvements to the aggregation process could be explored via the heating protocol as a sample's temperature history can affect rheological properties (*37*).

Successful completion of the hydrogel design and characterization enabled advancement to preliminary ex vivo and in vitro performance and safety assessments. Hydrogel plugs maintained IOPs greater than normal physiological IOP, suggesting the possibility of prolonging the injury-to-operation window, but doing so with a reversible sealant. In addition, the non-Newtonian state of $N_{95}BA_5$ allowed it to adapt to wound irregularities and also exhibit self-healing characteristics. This resilience has been noted in other noncovalent hydrogels, supporting the idea that these moldable materials can exhibit significant mechanical strength (*21*). In contrast, the NT comonomer proved less effective at maintaining high IOPs in the ex vivo test protocol, a result that paralleled reports of *n*-butyl cyanoacrylate scleral sealants preventing high IOP leaks in similar models (*38*).

The in vivo study provided preliminary insight into the hydrogel's efficacy in occluding open globe injuries. Whereas control groups approached 30% of normal pressure, treatment group IOP reached as high as 60%, which was corroborated by negative Seidel tests. The hydrogel formed a barrier capable of improving IOP during a 72-hour wait time for treatment.

Our preliminary biocompatibility assessment of $N_{95}BA_5$ suggests no significant adverse effects. Most reported studies on PNIPAM biocompatibility for biomedical and cosmetic applications support our findings (*39*–*42*); however, there has been one report of some observed tissue inflammation (*43*) and another reporting IOP decrease (*44*). No such history of testing exists for the hydrogel studied here, but PINIPAM copolymerized with BA has demonstrated basic cell compatibility (*45*). Our examinations demonstrated no neurotoxicity, no retinal tissue degradation, and no significant chronic inflammatory response after sustained exposure (30 days). Study limitations have now been addressed in this paragraph, and after an exhaustive literature review, we are not aware of any further material limitations. This preliminary demonstration of safety still requires a larger study size to rule out effects on retinal function.

Clinical user feedback is a valuable element in developing new interventions and therapies, and here, we specifically targeted military clinicians, who have encountered or who will encounter ocular trauma under conditions where temporary intervention may be preferable over full intervention. Although full intervention at first admission is preferred (*8*), some scenarios, such as mass casualty events, may create scenarios where multiple ocular injuries may delay intervention (*7*). The feedback captured from the military clinicians provided some good validation that the ease of use and the relevance of the technology were both real.

This study establishes foundational feasibility, safety, and efficacy of $N_{95}BA_5$ as a reversible thermoresponsive hydrogel for temporary closure of scleral perforations. This tissue repair technology sustained IOPs five times greater than the physiological range in bench testing of maximum adhesion, prevented hypotony for 72 hours after scleral trauma, and demonstrated sufficient biocompatibility for exposures longer than the intended use period. Furthermore, the hydrogel's adaptability to wound shape and unique deployment method make a unique and easy alternative to conventional methods.

MATERIALS AND METHODS

Study design

The overall objective of this study was to assess the feasibility of developing a temporary ocular repair technology with physical and mechanical properties suited to improving outcomes for globes compromised by scleral tears. This objective was segmented into three efforts: a polymer chemistry investigation of hydrogel properties correlated to synthesis route and composition, a materials science study of performance characteristics using benchtop models of ocular trauma, and an in vivo, preclinical study of safety and performance in an animal model of ocular trauma. Our initial work evaluated the potential of two compositions at different concentrations. These copolymers incorporated hydrophobic monomers (NT and BA) with NIPAM to form a temperature-responsive hydrogel in water with improved mechanical strength. Preliminary evaluation consisted of transition temperature's shift verification by scattering intensity, followed by examination of storage and loss moduli for relative viscoelastic properties. Identification of $N_{95}BA_5$ as the preferred sealant was followed by compression, tension analysis, and tissue adhesion tests at eye temperature. Secondary evaluation consisted of in vitro and ex vivo testing of different concentrations of the $N_{95}BA_5$ hydrogel in appropriate porcine test models. These tests led to further narrowing of the potential candidate compositions to only the 30% (w/w) $N_{95}BA_5$ for advancement to in vivo performance and safety testing.

The sealant technology safety and performance were assessed in an in vivo model of ocular trauma. A 2:1 (treatment/control) randomized and unblinded study was designed to evaluate the hydrogel sealant against the envisioned standard of care (no intervention). All animals (n = 18) received a 3-mm full-thickness laceration through the sclera, and treatment arm animals (n = 12) received the hydrogel sealant. All animals had regular IOP measures taken in triplicate at each time point to calculate a mean IOP for each time point. Animals were followed to one of three study endpoints (48 hours, 1 week, and 4 weeks), after which eyes were enucleated and fixed for histological preparation and analysis. Tissue segments were examined for evidence of adverse tissue responses including chronic inflammation, retinal degradation, cytotoxicity, and neurotoxicity.

Homopolymer and copolymer synthesis

PNIPAM, copolymer of NIPAM and NT ($N_{85}NT_{15}$), and copolymer of NIPAM and BA ($N_{95}BA_5$) were synthesized using free radical polymerization (*44*, *45*). For $N_{85}NT_{15}$, a solution of NIPAM (4.25 g), NT (0.75 g), and 2,2′-azobisisobutyronitrile (0.021 g) was dissolved in 60 ml of dry tetrahydrofuran (THF). The magnetically stirred solution was degassed, heated to 50°C for 24 hours under positive nitrogen pressure, and

allowed to cool. The reaction mixture was filtered (0.45-μm Teflon filter), and the filtrated volume was reduced by half. Ether was added with mixing to precipitate the copolymer. The precipitate was filtered off, washed with ether, and dried under vacuum to yield dry 4.64 g of copolymer product. For $N_{95}BA_5$, we followed the same procedure except that we used a different ratio of NIPAM (4.75 g) to BA (0.25 g). Using different ratios of THF/benzene as a solvent, we were able to synthesize homopolymers and copolymers with various molecular weights and polydispersities. After differentiation of the hydrogel compositions and the identification of $N_{95}BA_5$ for further application, the copolymer was then purchased from Sigma-Aldrich.

^{1}H NMR

Feed ratio accuracy was confirmed by ^{1}H NMR (Varian VNMRS-600). We prepared 5% (w/w) solutions of $N_{85}NT_{15}$ and $N_{95}BA_5$ in $CDCL_3$. Peak integration ratios were compared to theoretical ratios for compositional verification.

Hydrogel solution preparation

The required amount of PNIPAM, $N_{85}NT_{15}$, and $N_{95}BA_5$ was weighed for various concentrations of hydrogel aqueous solutions, from 0.8 to 43.2% (w/w). The powder was added directly to the sterile water. The vial containing the polymer suspension was then processed with a Misonix Sonicator 3000 using a cup horn high-intensity ultrasonic water bath at the maximum power setting (10) due to sample viscosity. A circulating temperature control water bath held at 2°C prevented sample heating due to prolonged horn activity. The sample was sonicated until a transparent clear hydrogel was obtained. The required sonication time ranged from 1 to 30 hours and depended on the hydrogel concentration and molecular weight of the homopolymers/copolymers.

Scattering intensity analysis

Scattering intensity measurements of 5% (w/w) hydrogels were carried out using a Photon Technology International Quantamaster fluorescence spectrophotometer. All intensity measurements were performed at an excitation wavelength (λ_{ex}) of 450 nm, with detectors positioned 90° from the light source. Emission spectra were recorded with a slit width of 0.1/0.1 nm. Quartz cuvettes (1 cm × 1 cm × 3 cm) containing the sample were placed in a cell holder, which was electrothermally controlled at a precise temperature regulated by a Peltier cooler. Each hydrogel was left under undisturbed conditions for 20 min at each temperature to obtain thermodynamic equilibrium.

Because the input light source was directed at the sample, the particles redirect the light away from a direct course through the cuvette with increasing severity as particle size increases. For small particle sizes, most of the input beam traverses the sample directly, resulting in small scattering measurements. As particle size increases, more of the light affects the particles, resulting in sideways diffusion of light that could be focused and then received by the sensor at 90° to the input beam. Scattering values were then normalized. Because of increase in sample opacity above LCST, most of the input beam was eventually reflected upon initial contact with the sample. As a result, higher temperatures saw a slow decrease in intensity because less input beam reached the center of the sample to be scattered toward the receiver.

Rheological analysis

An Anton Paar Modular Compact Rheometer (MCR) was used to measure the rheological properties of hydrogels. Eight milliliters of 10% (w/w) $N_{95}BA_5$ and $N_{85}NT_{15}$ hydrogel solutions was placed into the cylinder with special care to avoid evaporation of water. First, the hydrogels were investigated with strain-amplitude sweeps below their LCST (6°C), at LCST, and above the transition temperature (32°C) at constant angular frequency (10 rad s^{-1}). After the critical strain was found for each hydrogel, oscillation tests were performed to measure the loss (G'') and storage (G') moduli at a designated temperature (6°, 24°, and 32°C) using a circulating temperature control water bath. Strain was fixed at 0.1% (below the critical strain), and moduli were measured as a response to logarithmic angular frequency ramp from 0.1 to 1000 rad s^{-1}. Temperature-dependent changes in complex viscosity were performed using a fixed angular frequency of 10 rad s^{-1}, 0.1% strain, and a heat rate of 0.5°C/min on 5, 10, 20, and 30% (w/w) $N_{95}BA_5$ samples.

Compression test

To perform compression testing, liquid hydrogels were injected into a 17-mm × 17-mm × 5-mm glass mold and then heated to 32°C using a temperature-controlled Instron 5567 mechanical tester. After a 2-min curing period, the glass mold was removed, and the probe was placed in contact with the top of the molded hydrogel. The samples were then compressed at a rate of 1 mm/min. A stress-strain curve was obtained, and compressive modulus was determined as the slope of the linear region corresponding to 5 to 15% strain. The number of hydrogel samples was three per group.

Tensile test

Liquid hydrogel samples were shifted to the solid phase (32°C) using a hot plate and were detached from a metal mold. Samples were then blotted dry and fixed by two clamps of an Instron 5942 mechanical tester. The solid samples were stretched at a constant rate of 1 mm/min under controlled temperature. Young's modulus was determined as the slope of the linear region of the stress-strain corresponding to 0 to 10% strain.

Hydrodynamic light scattering analysis

Intensity distribution and R_H of 5% (w/w) $N_{95}BA_5$ aggregates were measured by the automated DynaPro Plate Reader II (Wyatt Technology). The DLS equipment is equipped with a thermostat-equipped sample chamber to maintain desired temperatures within a range of 4° to 85°C with great accuracy. A bubble-free sample of about 80 μl was introduced in a square glass cuvette through a micropipette. A drop of mineral oil (20 μl) was placed on the top sample solution to prevent water evaporation. Then, the sample cell was placed in the sample chamber of the DLS instrument and kept at constant temperature for 30 min, a procedure repeated for all desired temperatures to ensure thermodynamic equilibrium. This instrument measures the movement of particles under Brownian motion and converts this motion into size by using the Stokes-Einstein equation, as given below,

$$D = kT/6\pi\eta R_H$$

where k is the Boltzmann's constant, T is the absolute temperature, η is the viscosity, and D is the diffusion coefficient. All data were obtained by the instrumental software.

Ex vivo IOP measurement

Fresh (harvested within 24 hours) porcine eyes (Sierra for Medical Science) were mounted into a Styrofoam fixture and immobilized with dissection pins. Partial core vitrectomies were performed on each eye using the Constellation Vision System vitrectomy console (Alcon Inc.), and

IOP was measured by a digital pressure sensor inserted in the vitreous cavity (posterior segment of the eye) (Fig. 3A). A single 3-mm linear incision was created in the sclera, about 3-mm distance radial from the limbus, with the incision path running tangential to the limbus perimeter. An infusion cannula was placed in the vitreous cavity on the opposite side from the incision to supply a saline solution of about 37°C. Saline solution was infused either from a gravity-fed saline drip bag or from a digitally actuated infusion system. Partial core vitrectomy allowed faster diffusion of the liquid through the vitreous cavity, and saline ejection from the incision site confirmed incision success. Once confirmed, the infusion line was clamped to limit leakage during the test. The eye surface was dried with swabs to enable clean placement of the test substance (Fig. 3A). After this process, the IOP was gradually raised from baseline by manually increasing the infusion pump pressure until leakage was observed or the pressure sensor value no longer increased with increasing infusion rate (indicative of a nonvisible leak). The maximum pressure held (in millimeters of mercury) was recorded.

In vitro adhesion test

We prepared a range of 0.8 to 43% (w/w) PNIPAM and 30% (w/w) $N_{95}BA_5$ hydrogels for uniaxial adhesion testing. Fresh (harvested within 24 hours) porcine eyes (Sierra for Medical Science) were dissected into a 2-cm × 2-cm square shape. Hydrogel was compressed between two pieces of dissected scleral tissue fixated to the base and actuator arm of the pull tester (Fig. 3F). Apposed tissues were put into contact and pressed together using 15*g* of pressure for 2 min. The actuator then performed a pulling motion until the two samples separated, compromising the gel adhesion.

Deployment tool development and use

To deploy the hydrogel in the eye, a novel tool was developed as follows: an 18-gauge intravenous catheter tip trimmed to 5 to 8 mm was attached onto the Luer-Lock end of the 1-cm^3 syringe, which was inserted through the opening of the 15-cm^3 syringe. The space between the external wall of the 1-cm^3 syringe and the internal wall of the 15-cm^3 syringe was filled with ammonium nitrate and water. The endothermic dissolution reaction for the cooling mechanism was calibrated by using a pair of digital thermocouples to track the temperature in the jacketing chamber and inside the hydrogel chamber when different quantities of water and ammonium nitrate were mixed together. A working range of about 7 to 15 ml of water mixed with 6 to 8 g of ammonium nitrate would result in a temperature-lowering profile meeting the performance criteria. For testing and use, the tool components were first sterilized, and then, a sterile 1-cm^3 syringe loaded with hydrogel was loaded into the jacket. The jacket was loaded with ammonium nitrate and capped with a rubber vial cap. When ready for use, a separate syringe filled with sterile water and capped with an 18-gauge needle was inserted through the rubber vial cap, and water was injected into the jacket chamber. The tool was shaken a few times vigorously to initiate the reaction. After 60 s, the gel was ready for application.

Hydrogel preparation for in vivo validation

The 30% $N_{95}BA_5$ hydrogel used for in vivo characterization was sterilized by first measuring a prescribed quantity (1.0 g) of the dried powder form into a 10-cm^3 glass crimp vial container. Each open and filled container was placed inside a Tyvek sterilization pouch with the associated stopper and crimp top. The pouch was then sealed and ethylene oxide–sterilized. Still inside the sealed and sterilized pouch, the stopper and crimp top were manipulated on top of the open container, and the container was crimped shut inside the pouch. Once sealed, the pouch was opened, and the jar was withdrawn, leaving a sealed crimp container with 0.5 g of sterile hydrogel powder. The sterilized hydrogel powders were hydrated by injecting sterile water into the vial and performing the hydration procedure described previously. Once hydrated, the containers were transferred to a refrigerator for storage until use.

In vivo study design

An unblinded, two-arm randomized (2:1, treatment/control) study was conducted, in which all animals received 3-mm full-thickness incisions in the sclera of the right eye (OS) about 3 mm posterior to the limbus in the temporal superior quadrant to mimic a traumatic injury. The fellow eye (OD) in all rabbits was left untouched as a control. Treatment group animals received the hydrogel intervention. Control group animals received no intervention (current standard of care). Pigmented New Zealand rabbits were used for this study because the eye dimensions closely approximate the size of the human eye, thus mimicking approximate conditions for human use of the system.

For treatment group rabbits, once the open globe injury was created, the hydrogel-loaded syringe was prepared for use, as described previously. About 0.1 to 0.3 cm^3 of hydrogel was used for each procedure. Control group rabbits received the same surgical procedure to the right eye but received no intervention. At the end of each procedure, all rabbits in both groups received (quantity) subcutaneous injections of buprenorphine with repeat injections at 12-hour intervals for 48 hours. The research team regularly monitored each rabbit for the first 6 hours after the procedure. They then performed checkups at 6-hour intervals through the first 12 hours, followed by 12-hour interval checkups through the first week. At each 12-hour follow-up, rabbits were removed from their cage, and their eyes were visually inspected by the research team for any evidence of adverse tissue responses, such as swelling, inflammation, or bleeding from the sclera, conjunctiva, or other surrounding tissues, with instructions to notify the ophthalmologists of the team of any adverse events.

In vivo IOP measurements

IOP of both eyes of all animals was measured using a magnetically actuated veterinary rebound tonometer (Tonovet). Baseline IOP values were established by measuring IOP of each eye twice daily (a.m. and p.m.) for 5 days leading up to the procedure (Fig. 5D). The Tonovet calculates an average reading from six tonometric measurements taken in succession. Four successive readings were taken on each eye; thus, 24 measures contributed to the averaged IOP for each eye. For all tonometry measures, rabbits were removed from the cage and placed on an evaluation table for 2 min to allow the animal to relax. Stress from handling is known to artificially elevate blood pressure and IOP values. The average of three IOP measurements was recorded at each time point for each eye (both OS and OD) at regular intervals after the surgical procedure. Normalized IOP values were reported by dividing IOP_{OS} by IOP_{OD} to reduce the impact of confounding systemic effects (stress, infection, and medications). All surgical procedures were performed in the a.m. and completed before noon, thus allowing IOP measures to resume in the late p.m. of the same day.

In vivo study endpoint

Study endpoints for the rabbits were set at 48 hours, 1 week, and 4 weeks to evaluate the progression of the tissue response at the implant site. Rabbits were not followed longer than 4 weeks because the intended use of the hydrogel will be for less than 30 days. Rabbits were euthanized

by first administering a heavy dose of ketamine/xylazine anesthesia, followed by intravenous injection of a lethal dose of sodium pentobarbital via the auricular vein. Once euthanized, a surgical procedure was performed to quickly enucleate and fix the study eyes (OS).

In vivo histology analysis

Enucleated eyes were fixed in Davidson's solution to preserve structure of the total globe. Tissues were sectioned and stained with either hematoxylin and eosin (H&E) or Masson's trichrome stain to evaluate local inflammatory response and characterize fibrosis. Retinal detachments were assessed by histological evaluation of the posterior segments at study endpoints. Retinas were analyzed for evidence of photoreceptor outer segment disorganization or complete degeneration, indicative of retina separation from the choroidal vasculature and nutrient supply. In addition to evaluation of the overall structure, tissues were sent to an outside pathology laboratory (Comparative Bioscience Inc.) to be evaluated by a certified veterinary pathologist for evidence of cytotoxic or neurotoxic effects of the hydrogel on surrounding tissues.

Statistical analysis

For each animal at each time point, a normalized IOP measurement was calculated as IOP_{OD}/IOP_{OS} (Fig. 5E). The normalized measurement was statistically compared between treated and untreated groups using a generalized estimating equations model to account for correlated data arising from the repeated measures of IOP within an animal. The repeatedly measured normalized IOP was the dependent variable; independent variables were treatment group and time (day, a.m./p.m.) of measurement. The postsurgery treatment effect on IOP was tested over all postsurgical assessments, as well as at each measurement time. An omnibus test of treatment effect over postinjury time was tested by a score test of the main effect of treatment. Time-specific treatment effects were compared between groups with the addition of a day-by-treatment interaction term; treatment group differences were compared at each time point with a Wald test looking for a statistically significant difference between the two groups ($\alpha = 0.05$; two-sided). Mean (Standard Error of the Mean, SEM) outcome measurements were estimated and plotted for each day and treatment group. Eighteen animals randomized in a 2:1 fashion provided exceptional power (>95% for the treatment effect throughout follow-up time) to evaluate the mean group differences observed.

SUPPLEMENTARY MATERIALS

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Materials and Methods

Fig. S1. Schematic synthesis routes of the $N_{85}NT_{15}$ and $N_{95}BA_5$ copolymers.

Fig. S2. ^1H NMR spectrum for $N_{85}NT_{15}$ and $N_{95}BA_5$ copolymers in $CDCl_3$.

Fig. S3. Scattering intensity spectra of $N_{95}BA_5$ as a function of temperature.

Fig. S4. Strain amplitude for $N_{95}BA_5$ and $N_{85}NT_{15}$ at $T = 6$°C.

Fig. S5. Strain amplitude of $N_{85}NT_{15}$ at its LCST.

Fig. S6. Complex viscosity of $N_{95}BA_5$ as a function of temperature and concentration.

Fig. S7. Compressive stress-strain characterization and compressive modulus of the $N_{95}BA_5$ hydrogel.

Fig. S8. Tensile stress-strain characterization and tensile modulus of the $N_{95}BA_5$ hydrogel.

Fig. S9. Intensity distribution graph of DLS spectra for $N_{95}BA_5$ recorded at different temperatures.

Fig. S10. Hydrophobic/hydrophilic nature of the $N_{95}BA_5$ hydrogel above and below the LCST.

Fig. S11. Injector tool cooling reaction calibration curves for 2.5 and 12.5 g of ammonium nitrate to various volumes of added water.

Fig. S12. Visual evaluation of eye responses to surgical procedure and sealant placement.

Fig. S13. TRS in the hands of professionals: In vitro application at Walter Reed Medical Center.

Table S1. Injector tool design requirements.

Table S2. In vivo study tabulated trajectory.

Table S3. In vivo statistical analysis of average OD/OS for groups over time.

Table S4. Scleral tissue response at the hydrogel-sclera interface.

Table S5. Responses from freehand write-in section of user survey administered during clinical user workshop.

Table S6. Responses from multiple choice section of user survey administered during clinical user workshop.

Movie S1. In vivo trauma simulation and hydrogel application procedure.

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Science Translational Medicine

A reversible thermoresponsive sealant for temporary closure of ocular trauma

Niki Bayat, Yi Zhang, Paulo Falabella, Roby Menefee, John J. Whalen III, Mark S. Humayun and Mark E. Thompson

Sci Transl Med **9**, eaan3879.
DOI: 10.1126/scitranslmed.aan3879

A sealant to save sight

Traumatic eye injuries require rapid treatment to prevent deterioration of vision. As an alternative to suturing or adhesives, Bayat and colleagues developed a temperature-responsive synthetic hydrogel that acts as a temporary sealant. Testing the hydrogel in a model of open globe injury in rabbits showed that the sealant was easily deployed from a custom-designed temperature-controlled syringe device and preserved intraocular pressure without evidence of chronic inflammation or toxicity. After gelation, the sealant could be removed by exposure to cold water. In combat or low-resource settings, this hydrogel could close wounds temporarily to prevent further tissue damage or vision loss before surgery.

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(43) **Pub. Date: Aug. 4, 2016**

(54) **SYSTEM FOR SUTURELESS CLOSURE OF SCLERAL PERFORATIONS AND OTHER OCULAR TISSUE DISCONTINUITIES**

(71) Applicants: **John J. Whalen, III**, Pasadena, CA (US); **Niki Bayat**, Los Angeles, CA (US); **Yi Zhang**, Cypress, CA (US); **Paulo Falabella**, Newton, MA (US); **Mark E. Thompson**, Anaheim, CA (US); **Mark S. Humayun**, Glendale, CA (US)

(72) Inventors: **John J. Whalen, III**, Pasadena, CA (US); **Niki Bayat**, Los Angeles, CA (US); **Yi Zhang**, Cypress, CA (US); **Paulo Falabella**, Newton, MA (US); **Mark E. Thompson**, Anaheim, CA (US); **Mark S. Humayun**, Glendale, CA (US)

(73) Assignee: **UNIVERSITY OF SOUTHERN CALIFORNIA**, Los Angeles, CA (US)

(21) Appl. No.: **15/013,632**

(22) Filed: **Feb. 2, 2016**

Related U.S. Application Data

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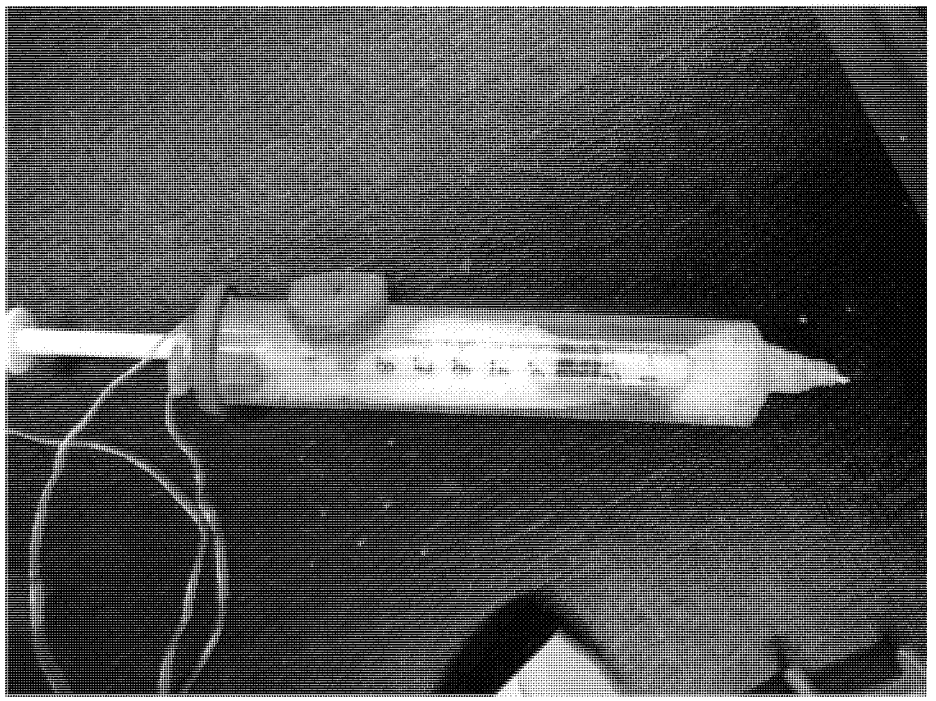
A61F 9/007	(2006.01)
A61L 24/06	(2006.01)

(52) **U.S. Cl.**

CPC ***A61L 24/0031*** (2013.01); ***A61L 24/06*** (2013.01); ***A61L 24/0015*** (2013.01); ***A61F 9/007*** (2013.01); ***A61F 9/00709*** (2013.01); *A61L 2430/16* (2013.01); *A61B 2017/005* (2013.01)

(57) **ABSTRACT**

The present disclosure describes, among other things, a thermo-responsive hydrogel comprising a PNIPAM copolymer having adhesive properties that are temperature dependent, as well as a device for administering the hydrogel, and methods for making and using the foregoing.



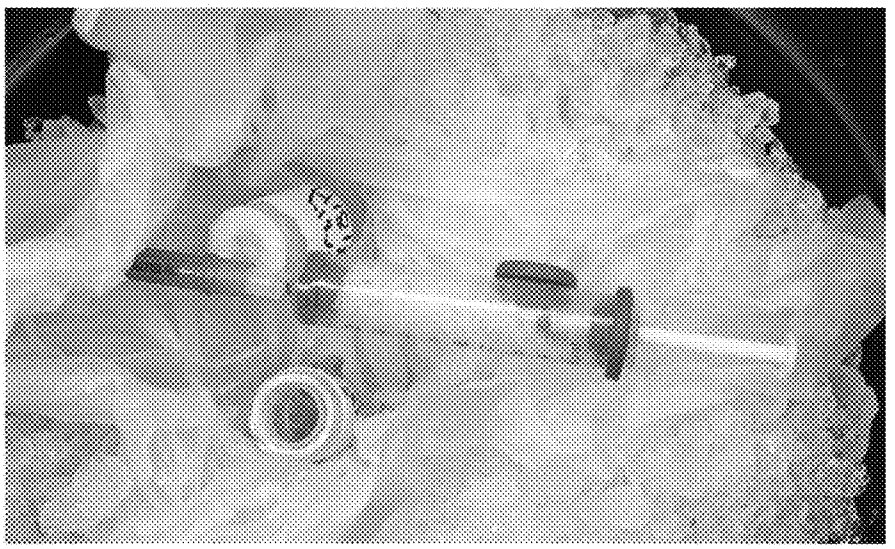


FIG. 1A

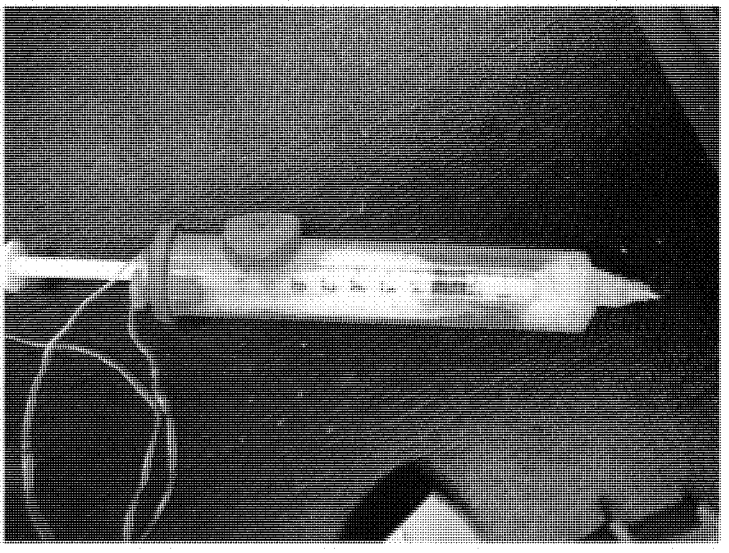


FIG. 1B

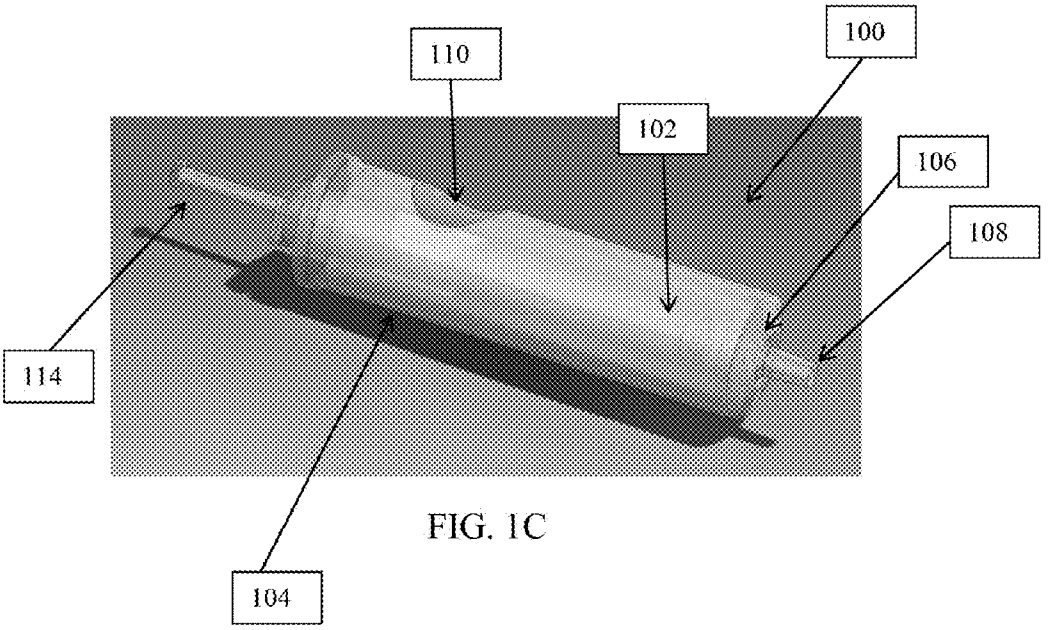


FIG. 1C

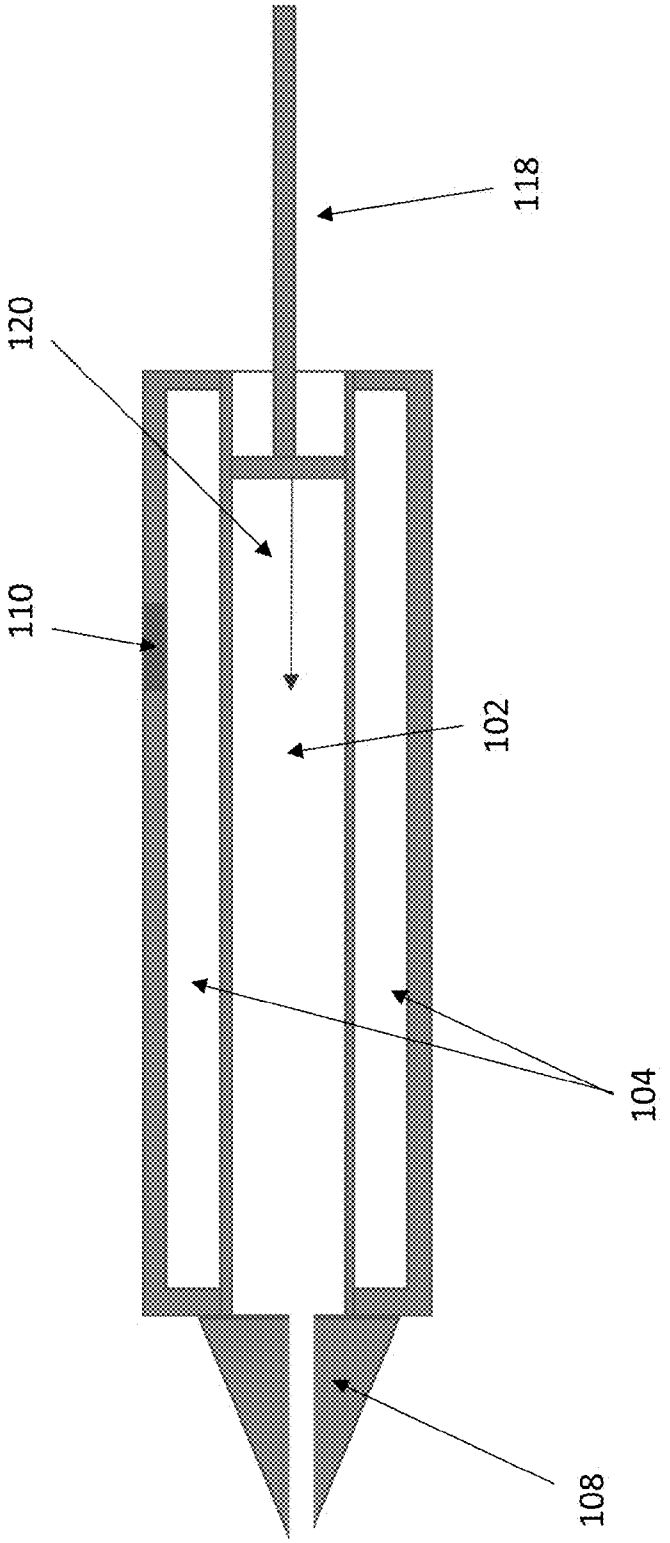


FIG. 1D

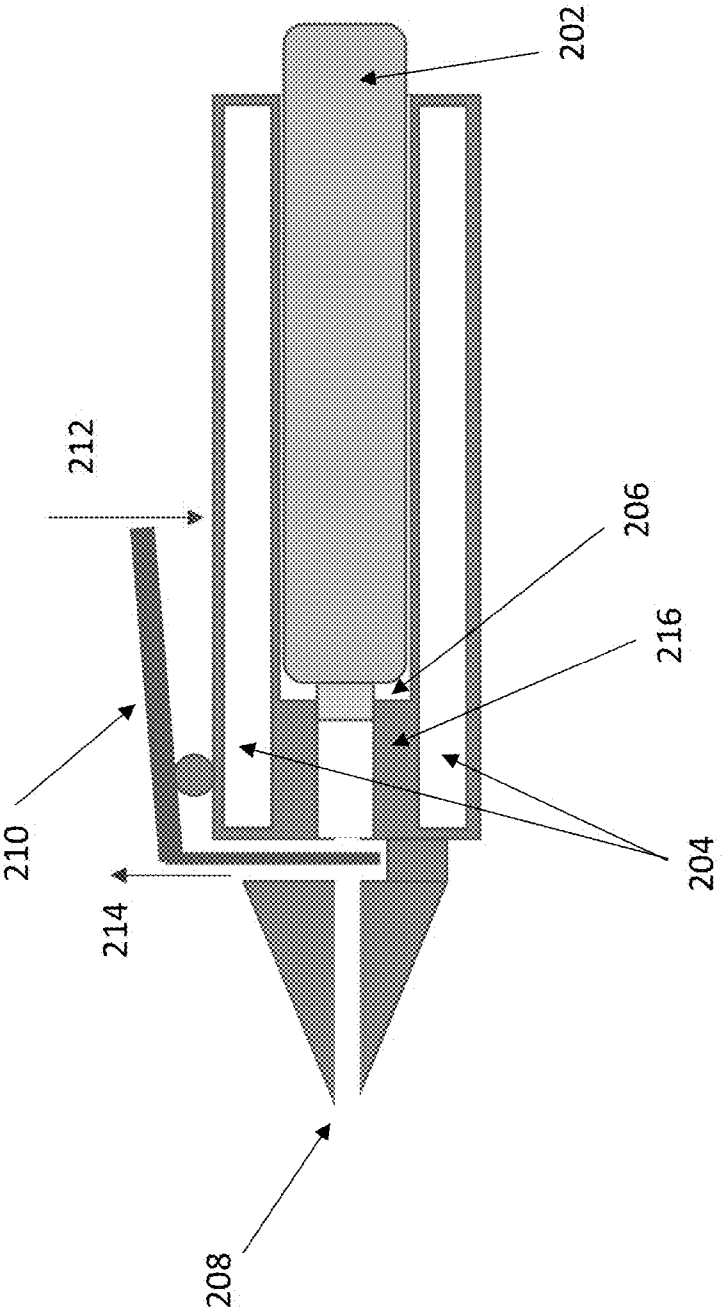


FIG. 2

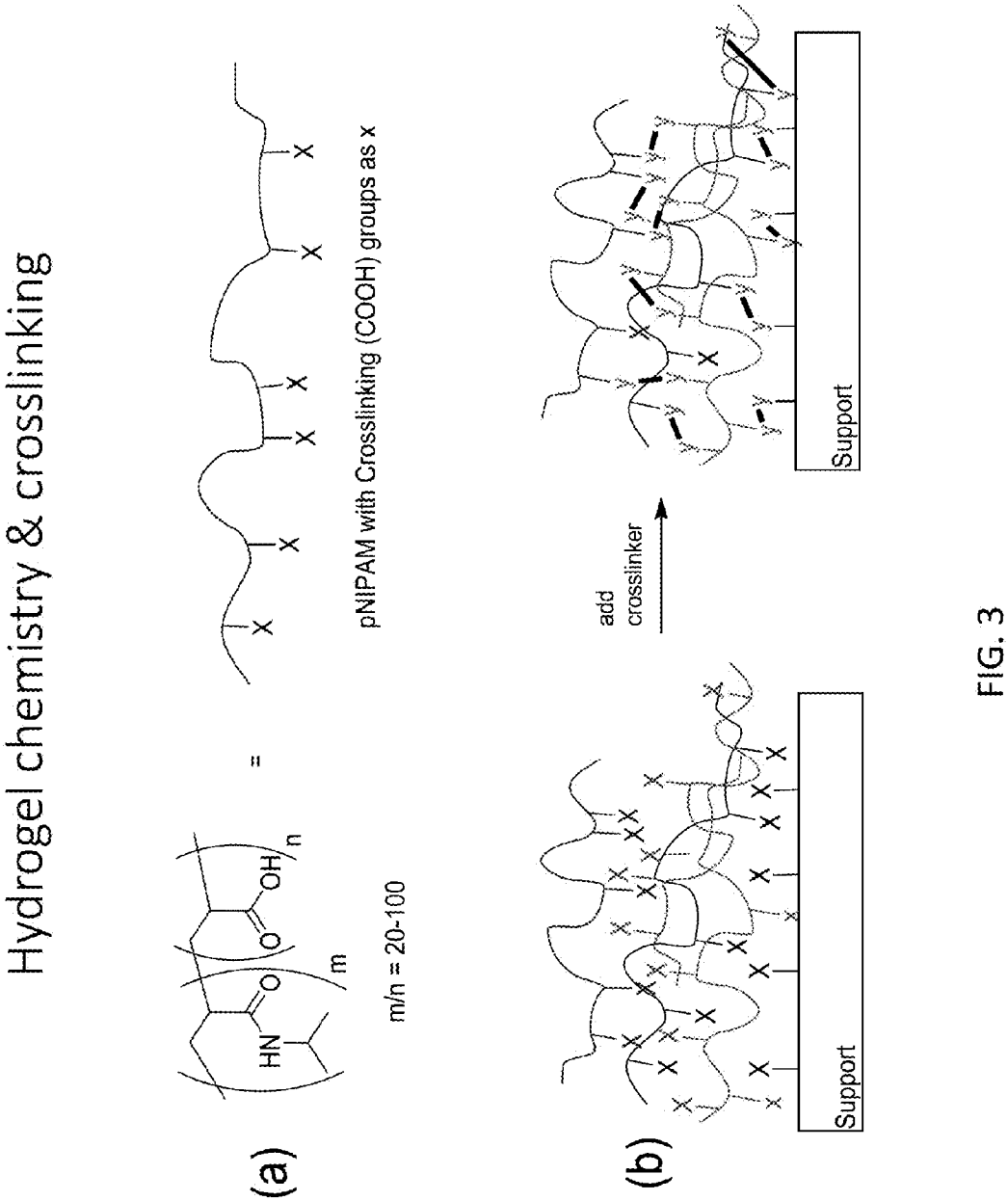

Hydrogel chemistry & crosslinking
(a)
=
pNIPAM with Crosslinking (COOH) groups as x
m/n = 20-100
(b)
add crosslinker
Support
Support

FIG. 3

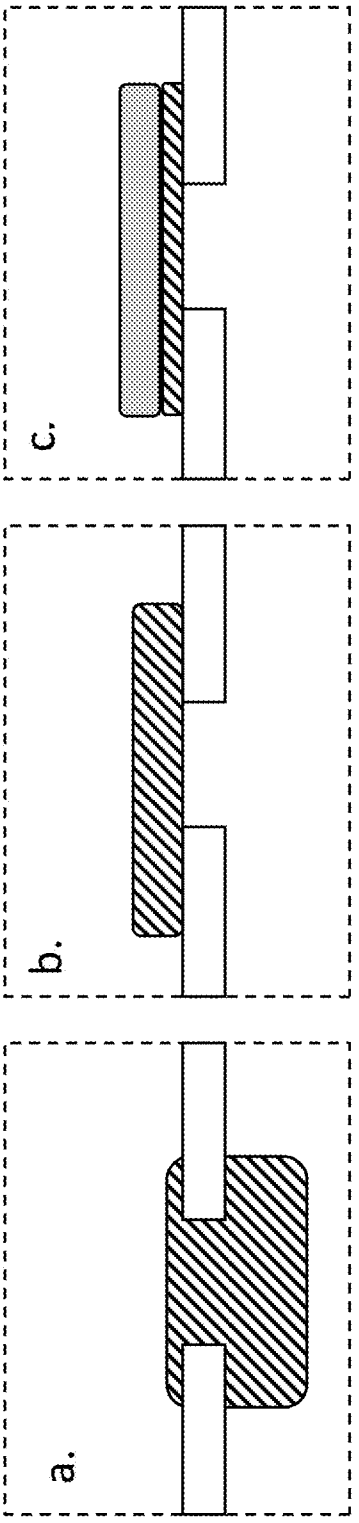


FIG. 4

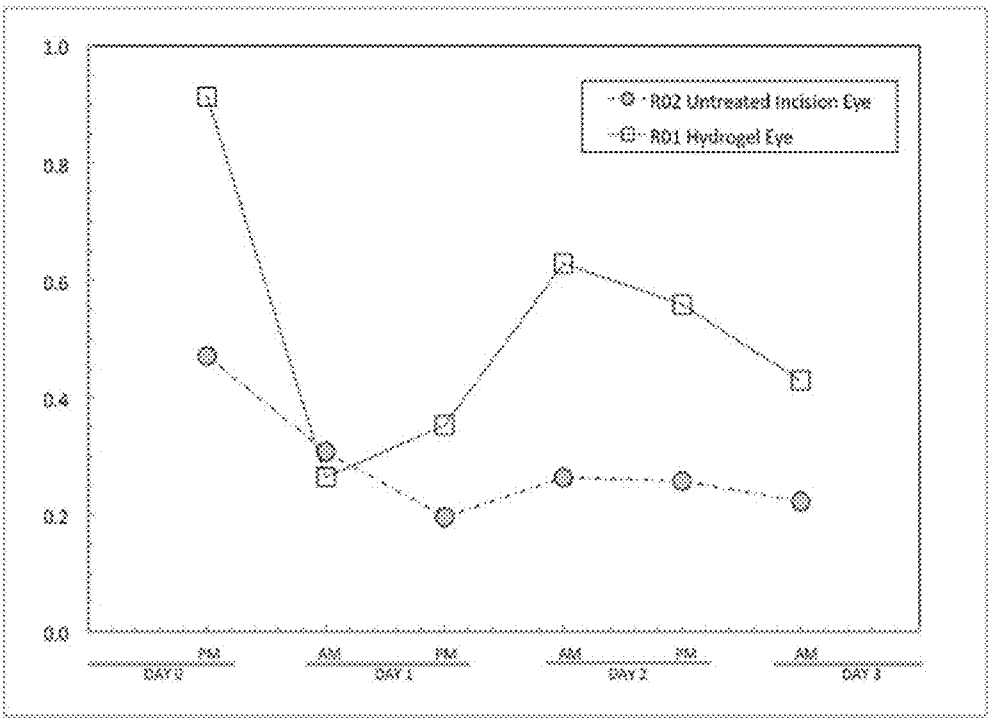

1.0
0.8
0.6
0.4
0.2
0.0
RO2 Untreated Incision Eye
RO1 Hydrogel Eye
PM
AM
PM
AM
PM
AM
DAY 0
DAY 1
DAY 2
DAY 3

FIG. 5

SYSTEM FOR SUTURELESS CLOSURE OF SCLERAL PERFORATIONS AND OTHER OCULAR TISSUE DISCONTINUITIES

CROSS-REFERENCE TO RELATED APPLICATIONS

[0001] This application is based upon and claims priority to U.S. Provisional Patent Application No. 62/110,851, entitled "System for Sutureless Closure of Scleral Perforations and Other Ocular Tissue Discontinuities" filed Feb. 2, 2015, attorney docket number 094852-0063.

[0002] This application is also related to U.S. Patent Publication No. US 2012/0109035 A1, published May 3, 2012, entitled "Reversible Adhesives" and Patent Cooperation Treaty Patent Publication No. WO 2009/097561 A1, published Aug. 6, 2009, entitled "Wound Closing Compounds with Additives." The entire content of each of these applications is incorporated herein by reference, including all exhibits.

STATEMENT REGARDING FEDERALLY SPONSORED RESEARCH

[0003] This invention was made with Government support under Contract No. W81X-WH12-1-0314, awarded by the Telemedicine and Advance Technology Research Center (TATRC) of the United States Army. The Government has certain rights in the invention.

TECHNICAL FIELD

[0004] This disclosure relates to, among other things, thermo-responsive hydrogel compositions, e.g., for treating ocular perforations.

BACKGROUND

[0005] Currently, in the United States, perforations of the eye wall (i.e., punctures through the entire wall) are closed using sutures. Sutures are placed through the layers of the eye wall tissue at the apposed margins of the perforation. The margins are drawn together and held closed with knots. Typically resorbable sutures are used, and therefore the suture knots may be exposed on the ocular wall surface for an extended period of time. Sutures leave high profile knots on the exterior surface of the eye. These knots can be felt by the patient and cause discomfort, which is known to lead to eye-rubbing and subsequent infection.

[0006] Outside of the U.S., certain bioadhesives are approved for use in the eye. For example, cyanoacrylate and fibrin glue are approved for use in Europe to close scleral and/or corneal perforations. These methods are currently not approved in the United States. Moreover, fibrin glue consists of genetically stripped fibrinogen and thrombin extracted from human or animal (e.g. bovine) blood. While this material is effective and biocompatible, fibrin glues carry a risk of viral and other pathogen transmission. Cyanoacrylate (e.g crazy glue) polymerizes in high modulus, rigid aggregates. The resulting solidified adhesive is very granular and often can feel like sand in the eye. This again can lead to discomfort and eye rubbing, which can cascade into irritation and infection. There is some evidence that unpolymerized cyanoacrylate may have some neurotoxic effects.

[0007] As noted above, both of the foregoing approaches have associated drawbacks. Provided herein are thermo-responsive polymers, hydrogel compositions, methods and devices which overcome many of the shortcomings of currently available materials and approaches for treating ocular trauma, especially under conditions requiring rapid and effective temporary treatment of ocular wounds.

SUMMARY

[0008] Generally, provided herein is a system for temporary closure of scleral perforations of the eye without the use of sutures. This system comprises a thermo-responsive polymeric gel whose adhesive and viscous properties are temperature dependent. Typically, the gel is adhesive and viscous near body temperature, and non-adhesive and non-viscous at room temperature. Also provided herein is a device for administering the polymeric gel, wherein the device comprises, e.g, components for properly manipulating and positioning the gel, controlling the temperature of the gel, and proper placement, i.e., delivery of the gel, as well as methods of making and using the polymeric gel and system.

[0009] In a first aspect, provided is a temperature-responsive hydrogel. The hydrogel comprises a poly(N-isopropylacrylamide) copolymer at a concentration of about 10 weight percent to about 60 weight percent in water, wherein the copolymer (i) is a copolymer of poly(N-isopropylacrylamide) and a second polymer that is either N-tert-butylacrylamide or butylacrylate, (ii) has a weight percent ratio of poly (N-isopropylacrylamide) to the second polymer of about 99:1 to about 50:50, and (iii) has a number average molecular weight of about 5,000 to about 5,000,000 daltons.

[0010] In one or more embodiments, the poly(N-isopropylacrylamide) copolymer is a poly(N-isopropylacrylamide): N-tert-butylacrylamide copolymer.

[0011] In one or more further embodiments, the poly(N-isopropylacrylamide) copolymer is a poly(N-isopropylacrylamide):butylacrylate copolymer.

[0012] In yet some additional embodiments, the weight percent ratio of poly(N-isopropylacrylamide) to the second polymer is selected from 99:1, 95:5, 90:10, 85:15, 80:20, 75:25, 70:30, 65:35, 60:40, 55:45 and 50:50. In some further embodiments, the weight percent ratio of poly(N-isopropylacrylamide) to the second polymer is in a range of about 95:5 to about 70:30.

[0013] In yet one or more further embodiments, the copolymer has a number average molecular weight of about 10,000 to about 3,000,000 daltons.

[0014] In yet some additional embodiments, the copolymer has a number average molecular weight of about 20,000 to about 2,000,000 daltons.

[0015] In some embodiments, the hydrogel has a copolymer concentration in water selected from the group consisting of about 10 weight percent, 15 weight percent, 20 weight percent, 25 weight percent 30 weight percent, 35 weight percent, 40 weight percent, 45 weight percent, 50 weight percent, 55 weight percent, and 60 weight percent in water.

[0016] In yet some further embodiments, the hydrogel has a lower critical solution temperature in a range of about 10° C. to about 35° C.

[0017] In some further embodiments, the poly(N-isopropylacrylamide) copolymer is a block copolymer.

[0018] In yet one or more additional embodiments, the hydrogel further comprises an excipient or additive.

[0019] In some embodiments, the hydrogel is in sterile form.

[0020] In yet further embodiments, the hydrogel has an adhesive strength in a range of about 10 mN to 10,000 mN when measured using an in vitro uniaxial adhesion test to scleral tissue at 37° C.

[0021] In some embodiments, the hydrogel comprises a bioactive agent.

[0022] In a second aspect, provided is an adhesive patch comprising a hydrogel according to any one or more of the aspects or hydrogel embodiments provided herein.

[0023] In some embodiments, the adhesive patch comprises the hydrogel deposited on a polymeric substrate. Illustrative substrates are, for example, selected from the group consisting of parylene, poly-lactic acid or co-polymeric matrices of poly-lactic and poly-glycolic acid, polyimide, liquid crystal polymer, and polydimethylsiloxane (PDMS).

[0024] In yet a further aspect, provided is a method for reversibly sealing an ocular perforation, the method comprising applying a hydrogel as provided herein to a tear in ocular tissue of a subject in an amount effective to seal the tear, wherein when exposed to a temperature above its critical solution temperature, the hydrogel becomes adhesive, and when exposed to a temperature below its critical solution temperature, the hydrogel becomes less adhesive.

[0025] In one or more embodiments related to the foregoing method, the hydrogel is maintained at a temperature below its critical solution temperature prior to said applying.

[0026] In some further embodiments of the method, the temperature of the ocular tissue is above the critical solution temperature of the hydrogel.

[0027] In yet some additional embodiments of the method, the hydrogel adheres to the tissue of the edges of the tear.

[0028] In some embodiments, the applying step comprises applying to an inner surface of an eye wall a slight excess of an amount of hydrogel effective to fill a void created by the ocular tear.

[0029] In one or more embodiments of the method, the ocular pressure is effective to press the excess hydrogel against the inner surface of the eye to thereby create an internal ocular seal.

[0030] In yet another aspect, provided is a device for delivery of a temperature responsive hydrogel, the device comprising (i) a first chamber for containing a temperature-responsive hydrogel, (ii) a second chamber at least partially surrounding the first chamber, said second chamber capable of maintaining a particular temperature or temperature range before and/or during delivery of the hydrogel, (iii) a port for delivery of the hydrogel from the first chamber to the delivery site, and (iv) a mechanism for delivery of the hydrogel from the first chamber to the delivery site.

[0031] In some embodiments of the device, the second chamber includes a cooling mechanism or material.

[0032] In some additional embodiments, the first chamber has a volume of about 0.1 mL to about 10 mL.

[0033] In one or more further embodiments, the size of the second chamber relative to the first chamber ranges from about 50:1 to about 10:1.

[0034] In some further embodiments, the second chamber further comprises a port for introduction of a coolant material.

[0035] In some further embodiments, the first chamber comprises a thermo-responsive adhesive hydrogel. In some additional embodiments, the first chamber comprises a thermo-responsive hydrogel comprising a PNIPAM-copolymer as provided herein.

[0036] In one or more additional embodiments, the second chamber comprises one or more materials effective to carry out an endothermic reaction.

[0037] In some embodiments, the one or more materials in the second chamber are sequestered from one another prior to reaction.

[0038] In some embodiments, the second chamber comprises ammonium nitrate. In an alternative embodiment, the chamber contains cooling elements that are electrically powered.

[0039] In one or more further embodiments, the device further comprises one or more tools effective to facilitate removal of a hydrogel plug from the eye.

[0040] In some additional embodiments, the device further comprises means for aspiration or irrigation of ocular tissue.

[0041] These, as well as other components, steps, features, objects, benefits, and advantages, will now become clear from a review of the following detailed description of illustrative embodiments, the accompanying drawings, and the claims.

BRIEF DESCRIPTION OF DRAWINGS

[0042] The drawings are of illustrative embodiments. They do not illustrate all embodiments. Other embodiments may be used in addition or instead. Details that may be apparent or unnecessary may be omitted to save space or for more effective illustration. Some embodiments may be practiced with additional components or steps and/or without all of the components or steps that are illustrated. When the same numeral appears in different drawings, it refers to the same or like components or steps.

[0043] FIGS. **1**A-**1**D provides illustrative embodiments of a device suitable for administering a reversible thermoresponsive hydrogel as provided herein. In some embodiments, the device is of a size suitable for use by a skilled practitioner, e.g., surgeon, using a single hand.

[0044] FIG. **2** provides an embodiment of a device for administering a thermally-responsive hydrogel using a pressure cartridge for storing and maintaining the hydrogel at a temperature suitable for manipulation of the gel.

[0045] FIG. **3** is a schematic illustrating modification of a PNIPAM copolymer with crosslinkable groups abbreviated as "X" (a), and crosslinking of the copolymer (b) to provide a crosslinked matrix that may also be crosslinked to a support. The crosslinking reaction leads conversion of x to y, with crosslinks illustrated by bold lines.

[0046] FIG. **4** is a schematic illustrating various patch styles including (a) an unsupported fluid hydrogel, (b) an unsupported patch hydrogel and (c) a supported patch hydrogel; and

[0047] FIG. **5** is a graph demonstrating results from Example 3 in which ocular pressure was plotted for rabbit eyes treated with PNIPAM-copolymer based hydrogels versus untreated eyes over a 72 hour period.

DETAILED DESCRIPTION OF ILLUSTRATIVE EMBODIMENTS

[0048] Illustrative embodiments are now discussed and illustrated. Other embodiments may be used in addition or instead. Details which may be apparent or unnecessary may be omitted to save space or for a more effective presentation. Conversely, some embodiments may be practiced without all of the details which are disclosed.

[0049] The singular forms "a," "an," and "the" include plural referents unless the context clearly dictates otherwise. Thus, for example, reference to a "water soluble polymer" includes a single water soluble polymer as well as two or more of the same or different water soluble polymers.

[0050] The term "about", particularly in reference to a given quantity, is meant to encompass deviations of plus or minus five percent.

[0051] The term "substantially" in reference to a certain feature or entity means to a significant degree or nearly completely (i.e. to a degree of 85% or greater) in reference to the feature or entity.

[0052] Where a range of values is provided, it is intended that each intervening value between the upper and lower limit of that range and any other stated or intervening value in that stated range is encompassed within the disclosure. For example, if a range of from 3 weight % to 10 weight % is described, it is intended that 3 mol %, 4 mol %, 5 mol %, 6 mol %, 7 mol %, 8 mol %, 9 mol %, and 10 mol % are also explicitly disclosed, as well as the range of values greater than or equal to 3 mol % and the range of values less than or equal to 10 mol %.

[0053] Generally, provided herein is a system for temporary closure of ocular perforations, i.e., punctures through the wall of the eye or other support structures of the eye. The system comprises, for example, a viscous polymer whose viscosity and adhesion to tissue is temperature dependent. Specifically, the viscosity and adhesion are suppressed at temperatures near room temperature (e.g. T=25° C.), while the polymer composition becomes viscous and adhesive at temperatures near physiological eye temperature (e.g. T=31° C.). Also provided herein are aspects related to, among other things: (i) an adhesive co-polymer, including a hydrogel comprising the co-polymer and its related properties; (ii) a method of placement and action by which the hydrogel composition is effective to seal an ocular perforation; and (iii) devices suitable for storing, delivering, and manipulating the adhesive during application and removal.

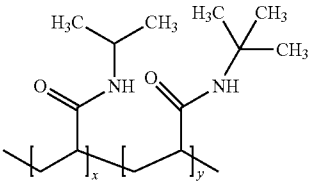
[0054] The instant adhesive ocular repair system (i.e., the co-polymer component, related hydrogel, delivery apparatus and related devices) is designed to temporarily occlude perforations of the eye wall (i.e. punctures through the entire thickness) and penetrations of the eye wall (i.e. insertions through a portion of the eye wall) to thereby prevent loss of intraocular pressure. Intraocular pressure is the isostatic pressure exerted by the fluid (vitreous humor) contained inside the eye. Leakage and loss of pressure can lead to retinal detachment, suprachoroidal hemorrhage, and subsequent permanent vision loss. Thus, the instant co-polymers, hydrogels, methods, and devices are useful for preventing any one of more of the foregoing conditions, among others.

Poly(N-isopropylacrylamide) Co-Polymer: Chemical Composition, Preparation and Characteristics

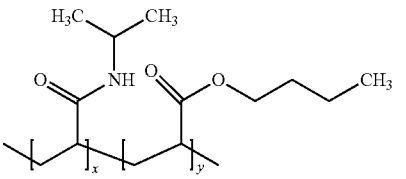
[0055] Poly(N-isopropylacrylamide) (pNIPAM) is a temperature-responsive polymer that exhibits a reversible macromolecular transition that demonstrates adhesive properties at body (eye) temperature and non-adhesive properties at decreased temperature. Provided herein are pNIPAM-based adhesive hydrogels effective to seal scleral wounds, among other things.

[0056] Copolymers for use in the compositions, hydrogels, methods and devices provided herein are PNIPAM-based copolymers. More specifically, provided herein is a copolymer of PNIPAM and a second polymer that is either N-tert-butylacrylamide (Formula I) or butylacrylate (Formula II). The copolymers were developed, at least in part, to provide formulations having improved adhesion to ocular tissue in comparison to formulations comprising PNIPAM alone. Generally, the copolymer has a weight percent ratio of poly (N-isopropylacrylamide) to the second polymer of about 99:1 to about 50:50. For example, the copolymer may comprise PNIPAM and the second polymer, i.e., either N-tert-butylacrylamide or butylacrylate, where the weight percentage of PNIPAM is in a range from 60 weight percent to 98 weight percent PNIPAM. The values of the subscripts x and y below will correspond to values effective to provide copolymers falling within the weight percentage ranges described above.

(I)



(II)



[0057] The PNIPAM-based copolymer may have a weight percent ratio of PNIPAM to the second polymer component (i.e., N-tert-butylacrylamide or butylacrylate) selected from 99:1, 95:5, 90:10, 85:15, 80:20, 75:25, 70:30, 65:35, 60:40, 55:45 and 50:50. In some embodiments, the weight percent ratio of poly(N-isopropylacrylamide) to the second polymer is about 95:5 to about 70:30. Generally, the copolymer will comprise a greater weight percentage of PNIPAM than of the second polymer, although in some embodiments, the copolymer may comprise equal percentages by weight of PNIPAM and the second polymer. Preferably, the copolymer comprises at least 50 weight percent or greater of PNIPAM, to thereby provide a copolymer having good adhesion performance, i.e., suitable for use in ocular applications.

[0058] The PNIPAM copolymer will typically possess a number average molecular weight of about 5,000 to about 5,000,000 daltons, or from about 10,000 to about 3,000,000 daltons, or from about 20,000 to about 2,000,000 daltons. Illustrative number average molecular weights include, for example, 5 kD, 10 kD, 15 kD, 20 kD, 25 kD, 30 kD, 40 kD, 50 kD, 60 kD, 70 kD, 80 kD, 90 kD, 100 kD, 200 kD, 300 kD, 400 kD, 500 kD, 600 kD, 700 kD, 800 kD, 900 kD, 1,000 kD, 1500 kD and 2000 kD, including all ranges between any two of the foregoing values.

[0059] Copolymers as provided herein can be prepared using known methods and available starting materials. See, e.g., G. G. Chen, A S Hoffman, *Nature* 1995-01-05, and A Gutowska, et al., *Journal of Biomedical Materials Research* 1995-07-01. An exemplary copolymer synthesis is provided herein as Example 1.

[0060] The instant PNIPAM:n-tert butylacrylamide and PNIPAM:butylacrylate co-polymers have been developed to

provide thermoresponsive hydrogel compositions having improved adhesion performance over hydrogels comprising PNIPAM as a single thermoresponsive polymer component. The instant copolymers were designed to provide polymers having a critical solution temperature that is lower than that of PNIPAM-alone. Introduction of a second copolymer component was effective to introduce a higher level of hydrophobicity into the hydrogel composition at body temperature, to thereby provide a PNIPAM copolymer having a balance of features, which when comprised within the instant hydrogels, are suitable for treating ocular tears, as well as for treating other ocular injuries or conditions.

[0061] Generally, the copolymers are block copolymers having properties particularly well suited for ocular applications. Representative hydrogels comprising the PNIPAM copolymers may comprise one or more of the following features:

TABLE 1

	PNIPAM:N-tert Butylacrylamide Co-Polymer	PNIPAM:Butylacrylate Co-Polymer
Preferred Chemical Formula:	$(C_6H_{11}NO)_x$:$(C_7H_{13}NO)_y$	$(C_6H_{11}NO)_x$:$(C_7H_{12}O_2)_y$
Co-Polymer wt % Ratio:	[99%:1%] to [50%:50%]	[99%:1%] to [50%:50%]
Preferred Number Average Molecular Weight Range:	5,000 to 5,000,000 (<800,000)	5,000 to 5,000,000 (<800,000)
Preferred Percent Aqueous Solution Range	10% to 60 wt % solids	10% to 60% wt % solids

[0062] The performance of the subject adhesive co-polymers is dependent, for example, on the lower critical solution temperature (LCST) of the co-polymer. The LCST is the temperature below which the solution becomes hydrophilic and less adhesive, and above which it becomes more hydrophobic and adhesive. Table 2 provides representative LCST ranges for the instant PNIPAM-based copolymers. Preferred polymers are those that become more viscous upon application to the environment of the eye. For instance, the copolymer may possess a LCST of 10° C., 11° C., 12° C., 13° C., 14° C., 15° C., 16° C., 17° C., 18° C., 19° C., 20° C., 21° C., 22° C., 23° C., 24° C., 25° C., 26° C., 27° C., 28° C., 29° C., 30° C., 31° C., 32° C., 33° C., 34° C., 35° C., 36° C., or 37° C.

TABLE 2

	PNIPAM:N-tert Butylacrylamide Co-Polymer	PNIPAM:Butylacrylate Co-Polymer
Desired LCST Range (° C.):	10° C. to 35° C.	10° C. to 35° C.

[0063] Hydrogels

[0064] The instant hydrogels typically comprise from about 10 wt % about 60 wt % PNIPAM copolymer in water. Illustrative copolymer concentrations in water include, for example, 10 weight percent, or 15 weight percent, or about 20 weight percent, or about 25 weight percent, or about 30 weight percent, or about 35 weight percent, or about 40 weight percent, or about 45 weight percent, or about 50 weight percent, or about 55 weight percent, or about 60 weight percent in water.

[0065] The hydrogel may also contain one or more excipients, stabilizers, additives or the like. The instant hydrogels may also comprise a bioactive agent, a diagnostic agent, a cosmetic agent, colorant (to enhance visualization), or any other agent suitable for delivery to the eye. For example, in one or more embodiments, the hydrogel may comprise a therapeutically effective amount of a bioactive agent. Representative active agents include but are not limited to, for example, antibiotics, anti-inflammatory agents, chemotherapeutic agents, steroids, and immunosuppressants.

[0066] Generally, the instant hydrogels possess several advantageous properties. For example, the instant hydrogels generally possess one or more of the following features. In some embodiments, the hydrogel is non-cytotoxic. In some other embodiments, the hydrogel is bioinert or biocompatible. In some further embodiments, the hydrogel is biodegradable. In yet some further embodiments, the hydrogel is inherently antibacterial.

[0067] The instant hydrogels will typically possess physical properties such that they can be tailored for use in an unsupported, fluid form or can be used in a more structured patch form (i.e., where the hydrogel itself provides its own mechanical structure and shape); alternatively, the hydrogel may be applied to a substrate whereby the hydrogel provides sealing/adhesive properties and the substrate provides mechanical support, shape and structure.

[0068] Additionally, the instant hydrogels are typically adhesive and viscous at body (e.g., eye) temperature (e.g., temperatures from about 30° C. to about 37° C.) or higher, or reach their final desired state at body temperature or higher. Favorably, the instant hydrogels can also be manipulated, positioned, and re-positioned at temperatures below body temperature.

[0069] In some embodiments, the hydrogel integrates chemistries that facilitate identification of fluid leakage in the eye.

[0070] Patches

[0071] As described above, the hydrogel may be provided in supported form, that is to say, deposited on a substrate. In one or more embodiments, the substrate is a flexible substrate also referred to herein as a backing layer or support. FIG. **4** provides schematic illustrations of various patch styles including (a) an unsupported fluid hydrogel (diagonal lines, e.g., filling an ocular void or tear), (b) an unsupported patch hydrogel (e.g., placed over an ocular tear or void) and (c) a supported patch hydrogel, where the hydrogel is situated upon a support. In one or more embodiments as illustrated in FIG. **4**(*a*), the hydrogel is an unsupported fluid material which plugs the penetrating injury. In one or more embodiments as shown in FIG. **4**(*b*), the hydrogel is a more structurally defined patch placed over an ocular injury. In such embodiments, the patch uses its adhesive properties to maintain a seal around the margins of the injury. Additionally, the mechanical integrity of the hydrogel is greater than in its fluid form, and can thus hold the margins of the injury together. In one or more embodiments as provided in FIG. **4**(*c*) the hydrogel is fixed onto a supportive substrate. The substrate provides mechanical integrity and contributes to sealing performance. The hydrogel in this embodiment serves to form an adhesive seal between the substrate and the tissue surrounding the trauma.

[0072] In one or more embodiments, a patch is prepared by utilizing pNIPAM copolymers further modified with suitable crosslinkable functionalities or X-groups; such crosslinking groups are well known in the art. For example, the copolymer may be modified to comprise crosslinking groups at from about 1-5% of the sites on the polymer chain, indicated by "X" in FIG. **3**. By virtue of adding crosslinkable groups, X, the polymer chains together can provide the pNIPAM copolymer as a single, interconnected mass (FIG. **3**, (b)) by virtue of addition of a suitable crosslinker. The crosslinks are illustrated by bold lines and conversion of the X groups to "y" groups. For example, if the X-groups are COOH, they can be crosslinked with alkyl-diamines to form an interconnected network that spans the entire polymer film. Moreover, if the substrate is decorated with the same X-groups as the polymer, crosslinking will take place between the polymer and substrate as well. Chemistries for modifying substrate surfaces with functional groups suitable for covalent attachment are well known.

[0073] In some embodiments, the PNIPAM copolymer is deposited on a substrate. Exemplary substrates include parylene, poly-lactic acid, polyimide, and polydimethylsiloxane, among others. A preferred substrate material is parylene. Parylene may be surface modified with carboxyl, amino, hydroxyl, alkyl-halide and other suitable crosslinkable groups using known chemistries. See, e.g., Wahjudi, J. H., et al., *Journal of Biomedical Materials Research,* Part A, 2009, 89A (1), 206-214. In one or more embodiments, the crosslinking reaction that is effective to stabilize the pNIPAM-copolymer film may also be employed to anchor it to a support surface. An pNIPAM-copolymer film having an initial thickness can be made arbitrarily thicker by addition of a crosslinker dissolved in the polymer film. Heating or irradiating the film can promote a crosslinking reaction.

[0074] Illustrative crosslinking chemistries include, for example, use of a PNIPAM copolymer comprising a small number of 2-hydroxyethylacrylate groups, which can then be efficiently cross-linked with tartaric acid. The foregoing copolymer can also be cross-linked with other poly-functional carboxylic acids (malic, citric, malonic, succinic, glutaric adipic acid) using ester- or etherification or N,N'-methylenebisacrylamide. Diamines such as triethylenetetramine may be used to crosslink PNIPAM copolymers functionalized with monomers containing an active ester group such as N-hydroxysuccinimide (NHS).

[0075] In some embodiments, the support is at least partially flexible to allow for delivery at sites that are not flat. In some embodiments, the backing layer is sufficiently flexible to conform to a delivery site such as the eye. In some embodiments, the patch includes an optional release liner that covers the adhesive hydrogel prior to application at the delivery site. In preferable embodiments, the release liner is impermeable and/or non-reactive with the adhesive hydrogel.

[0076] Device

[0077] In yet another aspect, provided herein is a device for storing, manipulating and delivery of the instant hydrogels to ocular tissue. In one or more embodiments, the device may also be used for heating saline infusions. In some embodiments, the hydrogel is delivered to its desired target site with minimal release of excess copolymer.

[0078] In some embodiments, provided is a device that is capable of reducing the temperature of the hydrogel. In one or more further embodiments, the device is also capable of facilitating co-polymer detachment from tissue. In yet one or more further embodiments, the device additionally comprises manipulation features (e.g. stylus, probe, forceps, or scalpel, etc.) to assist in manipulating the hydrogel plug to facilitate removal. In yet some further embodiments, the device may include aspiration and/or irrigation capabilities to facilitate removal of the co-polymer.

[0079] Thus, in one aspect, a device for maintaining a desired temperature and for delivery of a reversibly thermally-responsive adhesive hydrogel is described herein. In general, the device includes a first chamber for containing the adhesive hydrogel and a second chamber at least partially surrounding the first chamber for holding the adhesive hydrogel at a particular temperature or temperature range before and/or during delivery of the adhesive hydrogel. The device also includes a port, opening or other structure for delivery of the adhesive hydrogel from the first chamber to the delivery site. Finally, the device includes structure and/or a mechanism for delivery of the adhesive hydrogel from the first chamber to an intended delivery site.

[0080] In one exemplary embodiment, as shown in FIGS. **1**A-**1**D, the device **100** includes a first chamber **102** that is at least partially positioned within a second chamber **104**. In the embodiments as shown in FIGS. **1**A-**1**D, the first chamber is nested within the second chamber. The first and second chambers are operatively connected in a suitable manner such that the first chamber is at least partially maintained within the second chamber. In the embodiments shown in FIGS. **1**A-**1**C, the first and second chambers are connected at a first end **106**. It will be appreciated that in some embodiments the first and second chamber may be formed of separate pieces that are mechanically attached or adhered. In other embodiments, the first and second chambers may be formed of a single piece. In some embodiments, the first and second chambers are formed of separate pieces that are adhered using a suitable adhesive. Suitable adhesives are known in the art and may be determined by requirements of the materials used for the first and/or second chambers. The size and/or volume of the first and second chambers may be selected as necessary for ease of use and/or the requirements of the delivery site. For example, in some embodiments, the device may be intended for use by one hand of a user. In this embodiment, the width of the second chamber and length of the device may be determined by the needs of holding the device. In some non-limiting embodiments, the device has a total length of up to about 6-10", about 6-9", about 6-8" or about 6-7". Similarly, the volume of the first and/or second chambers may be sized to hold a desired amount of material. In some embodiments, the first chamber is sized to contain at least about 0.25-5 mL or up to about 5 mL of the adhesive hydrogel. In other embodiments, the first chamber is sized to contain at least about 0.5-5 mL, about 1-5 mL, about 1.5-5 mL, about 2-5 mL, about 2.5-5 mL, about 3-5 mL, or about 4-5 mL.

[0081] The device further includes an opening, delivery port, or nozzle **108** for delivery of the adhesive hydrogel to the treatment area. The discussion hereafter is with reference to a nozzle, although it will be appreciated that the discussion is applicable to other embodiments of a delivery portion of the device as appropriate. As seen in FIG. **1**C, the nozzle is connected to the first chamber such that the adhesive hydrogel may flow from the first chamber, through the nozzle and to a delivery site. The length and diameter of the nozzle may be any suitable dimension as required for the delivery site. In some embodiments, at least the end of the nozzle has a gauge ranging from 10 gauge through 24 gauge in tube bore.

[0082] The device further includes a delivery mechanism **114** that effects delivery of the adhesive hydrogel from the first chamber **102**, through the nozzle **108** and to the delivery site. FIG. **1**D depicts an embodiment where the delivery mechanism is a plunger-type mechanism **118**. Movement of the plunger in the direction of arrow **120** pushes the adhesive hydrogel through the first chamber to the delivery site. In the embodiment as shown in these figures, the plunger is manually operated. However, it will be appreciated that the delivery mechanism may be operated by other means as known in the art including, but not limited to, remote operation using a processor. Preferably, the delivery mechanism provides a seal with the first chamber during storage and during delivery so that the adhesive hydrogel does not exit the first chamber at the opposite end from the nozzle. The nozzle may be formed of any suitable material known in the art that is suitable for delivery of the adhesive hydrogel. In embodiments, the material of the nozzle is selected from a material that is minimally thermally conductive. A material that is minimally thermally conductive prevents or minimizes an increase in temperature of the nozzle from contact with the delivery site. In some embodiments, the nozzle is formed from a plastic such as that used for IV catheters.

[0083] In some embodiments, the second chamber includes a cooling mechanism or material as described herein. In some embodiments, the second chamber includes an opening or port **110** allowing access to the second chamber. In embodiments, the cooling mechanism may be pre-loaded or the cooling mechanism may be a continuous cooling mechanism. A continuous cooling mechanism may include a recirculating coolant and means for cooling the coolant during circulation. The size of the second chamber may be any suitable size as required to contain the recirculating cooling system or, alternatively, to contain a suitable amount of coolant material. In some embodiments, the second chamber should be sized to contain an amount of coolant material that cools the adhesive hydrogel in about 3-5 seconds. In other embodiments, the second chamber should be sized to contain an amount of coolant material sufficient to maintain the temperature of the first chamber at about 0-10° C. for at least about 10 minutes. As one example, where the first chamber contains about 0.5 mL of an adhesive hydrogel, the second chamber should be sized to contain about 7-10 g of a cooling material such as ammonium nitrate and about 5-20 mL of water.

[0084] In some embodiments where the cooling mechanism requires addition of a reactant (e.g. addition of water to ammonium nitrate or vice versa), the reactant (or additional reactant) may be added to the second chamber through the port. The port may be open or resealable. In some embodiments, a plug **112** is used to seal the port. The plug may be removable and/or may allow for introduction of a material through the plug. In some embodiments, the plug is formed of a material that can be pierced to allow a reactant to be added (or removed) from the second chamber. In one non-limiting embodiment, the plug is a rubber plug that may be pierced for addition or removal of a reactant. In some embodiments, the second chamber is air-tight and/or liquid tight when the port is sealed by a plug.

[0085] Further in reference to the embodiment provided in FIG. **1**D, the device includes an internal hydrogel reservoir for storing the hydrogel prior to its use, which is surrounded by a jacketing reservoir/space designed to contain a system for regulating the temperature of the hydrogel reservoir. This jacket may be a volume space for filling with endothermic reactants or a cavity filled with cooling elements (e.g. electrical elements). In embodiments in which chemical reactions are used to cool the system, an access port may be required to introduce reactants. Examples of endothermic reactions that may be employed include ammonium nitrate and water; urea and water; and barium hydroxide, ammonium chloride and water.

[0086] One end of the long axis of the device may be fitted with a nozzle/needle for releasing/deploying the hydrogel from the reservoir into the target site. This tip should be thermally insulating either by design or materials selection or both. Access from the reservoir to the nozzle may be controlled by a gating mechanism, e.g., to prevent leakage or premature release of hydrogel and/or to control release during deployment. In some embodiments, the hydrogel may be driven out of the reservoir using a plunger type mechanism. This mechanism may be actuated manually by applying pressure to the plunger end by the user, or alternatively, may be automated by using a driver type mechanism, e.g. a spring loading mechanism, or a servo motor, or other subsystem.

[0087] FIG. **1**A is an image of an embodiment of a device for delivering a thermos-reversible hydrogel. In the embodiment illustrated, a 1 mL syringe serves as a reservoir for the hydrogel, while a 10 mL syringe serves as a jacketing reservoir, e.g., for cooling the hydrogel. In the exemplary embodiment, the jacketing reservoir is filled with ammonium nitrate. The ammonium nitrate can be placed into the reservoir via a loading port accessible for example, via a plug on the external cylindrical wall of the jacket as shown. In this embodiment, a sterile plastic catheter tip (covered with a cap at left) is used to transfer the hydrogel from the reservoir to the target tissue site. The entire unit may, if desired, be stored over ice prior to use to further accelerate cooling of the hydrogel.

[0088] FIG. **1**B illustrates an embodiment of the device wherein the device comprises integrated temperature sensors for monitoring the temperature inside the jacket and inside the hydrogel reservoir. In one or more embodiment, the device may comprise one or more temperature sensors for monitoring the temperature inside the jacket, or inside the hydrogel reservoir, or at both locations.

[0089] The device, and each separate piece of the device, may be formed of any suitable material including, but not limited to, metals, polymers, and plastic. The choice of material may be guided by whether or not the device is reusable or disposable, which typically requires the use of more cost effective materials.

[0090] Although, the devices as shown in FIGS. **1**A-**1**D are cylindrical, it will be appreciated that the device may have any suitable shape as needed. The device may further include grips and/or specific shapes to enhance holding of the device. For example, where the device is designed to use similar to a pen, the device may include rubber grips or areas positioned on the second chamber near the nozzle to enhance grip. In other embodiments, the device may be shaped or ergonomically designed to be used manually.

[0091] The device may further include sensors such as temperature sensors that monitor the temperature of the first chamber, second chamber and/or the nozzle. FIG. **1**B shows temperature sensor **116** to monitor the temperature of the first chamber.

[0092] In some embodiments, rather than requiring storage at cool temperatures, the hydrogel may be stored under pressure. In some embodiments, the adhesive hydrogel is stored under pressure in a suitable cartridge. For these embodi-

ments, the delivery device may be configured to deliver the adhesive hydrogel from a pressure cartridge. One suitable embodiment using a pressure cartridge is shown in FIG. **2**. In these embodiments, the cartridge **202** comprising the adhesive hydrogel under pressure is at least partially insertable into a cooling chamber **204**. As seen in FIG. **2**, the cooling chamber **204** has an open section for insertion of the cartridge **202**. The device may include a holder **216** for the maintaining the cartridge within the cooling chamber. The holder may include means for retaining the cartridge including, but not limited to, a threaded area for mating with the cartridge or a narrowed area that at least a portion of the cartridge may be inserted into for retention of the cartridge. In one embodiment, the cartridge is in an open configuration while in the holder.

[0093] The device further includes an actuator for releasing the adhesive hydrogel from the cartridge to the delivery site. One embodiment of a mechanical actuator **210** is shown in FIG. **2**. In this embodiment, the cartridge **202** is inserted into and retained by the holder **216**. The device may further include a piercing member that pierces or opens the cartridge during insertion of the cartridge into the holder. The actuator in a first position seals the cartridge or holder to maintain the pressure of the cartridge and prevent delivery of the adhesive hydrogel. Movement of the actuator to a second position allows release of the pressure and the adhesive hydrogel from the cartridge, through the nozzle **208** and to the delivery site. In one embodiment, the actuator includes a handle portion, a hinged portion, and a sealing portion. Movement of the handle portion in a direction as shown by arrow **212** results in movement of the sealing portion away from the cartridge and holder (see arrow **214**), resulting in delivery of the adhesive hydrogel.

[0094] In the embodiment provided in FIG. **2**, the pressurized container may allow for longer storage of the hydrogel and hydrogel release without a need for mechanical actuators to drive release. A gating mechanism is provided to control release of the pressurized hydrogel. As described above, the release port/injector tip is preferably fabricated from a thermally insulating material or is designed in such a manner as to minimize thermal conduction from the external environment to the lumen of the injector tip.

[0095] In one or more embodiments, the device allows for easy transportation, deployment and manipulation of a thermo-responsive, reversible adhesive for placement in or on a targeted site.

[0096] In one or more additional embodiments, provided is a device comprising a hydrogel reservoir, a jacketing reservoir surrounding the hydrogel reservoir which may be used to provide thermal control of the hydrogel reservoir contents, a dispenser tip from which the hydrogel is deployed from the reservoir to a targeted area, a gating mechanism which allows controlled of release of the hydrogel from the tip, and an actuation system which provides a driving force to move the hydrogel from the reservoir out through the tip.

[0097] In yet some further embodiments of a device, the actuation system is driven by a mechanical mechanism, or is driven by an induced pressure, or via an electro-mechanical system or other method for displacing hydrogel from the reservoir towards the outlet dispenser tip.

[0098] In some embodiments, the device may be modular with interchangeable parts, or alternatively, may be comprised of a single working unit.

[0099] In some embodiments, the device comprises parts which may come into a sterile field for operation, and that can be sterilized or are sterile.

[0100] In one or more embodiments, the device is reusable. Alternatively, the device may be for single use.

[0101] In some embodiments, the device is capable of measuring the amount of hydrogel deployed.

[0102] In some embodiments, the device can report/provide temperatures of the hydrogel inside the reservoir, or can provide targeted deployment site surface temperature, or both.

[0103] In a preferred embodiment, the device can be operated by a single user using a single hand; in an alternative embodiment, the device can by operated by a single user using both hands. In some further embodiments, the device can be operated using foot or other body-controlled actuators.

[0104] The device may allow the hydrogel to be stored in a usable condition for prolonged periods of time regardless of external environmental conditions (high temperature, low temperature, etc.). In some embodiments, the device is relatively lightweight, allowing for easy manipulation of the hydrogel during implantation.

[0105] In some embodiments, the device comprises a system for rapidly cooling and maintaining a cold hydrogel temperature for a period of time sufficient to allow proper adhesive deployment; the system can be electrically driven or provided by virtue of an endothermic reaction driven, or via another mechanism.

[0106] In some embodiments, the device is capable of delivery of an amount of hydrogel sufficient for carrying out a desired procedure without requiring refilling. The volume may be from about 1 mL of adhesive hydrogel to as much as 50 mL of hydrogel.

[0107] In some embodiments, the device complies with specifications which meet military medical requirements above and beyond normal specifications; examples include improved packaging, enhanced transportation testing specifications, and improved thermal stability, to name a few.

[0108] The device will generally, in one or more embodiments, include a dispenser, cannula, or needle that is thermally insulated to minimize thermal energy transmission from the external environment to the lumen of the tube to prevent premature hydrogel fixation.

[0109] In one or more further embodiments, the device can be sterilized without compromising its ability to operate.

[0110] In one or more further embodiments, the device allows for the hydrogel to be transferred from the reservoir to the dispenser for deployment in a sterile field.

[0111] In some further embodiments, the device is a plug-in style device which may be electrically wired, for example, for use in a surgical theater where full facilities may be present and a larger device could be stored, maintained and operated.

[0112] In one or more additional embodiments, the device incorporates plug in electronics to drive cooling or actuation for hydrogel release.

[0113] It will be appreciated that in some embodiments, the device may be useful for delivery of other thermosensitive materials.

[0114] Methods of Use

[0115] In one or more embodiments, the instant hydrogel is a free flowing thermo-reversible hydrogel adhesive that is effective to occlude penetrating injuries through tissue by placing a bolus of the hydrogel across the margins of the penetration to provide a mechanical occlusion. In some

embodiments, by virtue of becoming viscous at an elevated temperature such as body temperature or above, the hydrogel is effective to provide a physical or mechanical occlusion to the opening in the tissue.

[0116] In one or more further embodiments, a method is provided for adhering the hydrogel to an ocular tissue surface to thereby provide a seal to separate two regions which were previously connected by a penetration through the tissue.

[0117] In one or more additional embodiments, a method for delivering a hydrogel to an ocular tear is provided. For example, the hydrogel may first be being cooled down below its lower critical solution temperature (LCST), followed by application to an ocular tissue site. By cooling the hydrogel below its LCST, the hydrogel can be more readily manipulated and deployed, by virtue of its lower viscosity. Once in place, the hydrogel is allowed to raise above its LCST to thereby fix the hydrogel into position, via either adhesive fixation or mechanical fixation, or via another suitable method of immobilization. Typically, the instant hydrogels and methods are useful for closing linear perforation of about 3 cm or less, or of about 2 cm or less.

[0118] In yet an additional method, the reversibly adhesive hydrogel is applied as either a supported or unsupported patch over an area of compromised (cut, missing, penetrated, etc.) tissue to temporarily reestablish continuity of the tissue.

[0119] In one or more further embodiments, a method of applying a hydrogel is provided wherein temperature is employed to reversibly control the adhesion/occlusion characteristics of the hydrogel for a therapeutic effect. For example, heat is used to allow the hydrogel to become fixed upon application to a target site, and cooling is used to release the hydrogel from the target site at some point following its application, e.g., after it is no longer needed.

[0120] In yet one or more additional embodiments, the hydrogel may be released from its target site, e.g., ocular tissue, by reducing its local temperature, for example by applying a small stream of iced water to the hydrogel directly via an irrigation tool.

[0121] In one or more further embodiments, provided is a method to occlude penetrating injuries to tissues, for example, in the case of a penetrating injury to the wall of the eye (the sclera). Generally, the injury is first located and characterized to determine the likelihood that the hydrogel may be effective in addressing the problem. If application is via a device, device may be prepared for use (e.g. unpackaged, sterilized, etc.), and the hydrogel then allowed to reach its desired working temperature, e.g. cooled. Once at its working temperature, the hydrogel can be administered to the injury site. Once in place, the hydrogel may be afforded time to raise in temperature and transition above its LCST. Once the hydrogel becomes more viscous, excess material on the exterior of the placement site may be excised, e.g. using shears or a blade.

[0122] Thus, the instant hydrogels can be effective to seal an ocular tear by (1) filling a void created by the perforation with a mass of co-polymer, (2) adhering to the tissue of the margins (edges) of the perforation, and (3) injecting a slight excess of polymer at the inner surface of the eye wall, the ocular pressure presses excess polymer against the inner surface, creating an internal seal. Thus, the hydrogel once applied creates a "plug" that fills and self-seals the ocular perforation. Once in place, excess polymer on the exterior surface of the eye can be shaved away with a scalpel or other cutting device to create an ultra-low profile on the surface of the eye, thus minimizing discomfort.

[0123] In instances in which posterior segment surgery requires a procedure called a vitrectomy, in which the vitreous—a jelly like biological material filling the larger posterior chamber of the eye—is removed and substituted with room temperature saline via infusion, if the saline is at room temperature, this may delay or inhibit transition of the adhesive hydrogel from non-viscous to viscous.

[0124] The hydrogel, devices and methods provided herein are well suited to address problems associated with penetrating injuries to the eye. The eye consists of a firm, walled structure (the sclera) that creates the spherical boundaries of the eye. Inside of the sclera, the majority of the volume is filled with a jelly-like, transparent fluid, the vitreous humor. The interior, posterior wall of the eye is lined with the retina, the sensory tissue which is responsible for converting images observed by the individual into neural signals which are then transmitted to the brain via the optic nerve. The interior of the eye is under pressure with respect to the exterior of the eye, created by continuous secretion of aqueous humor in the anterior segment of the eye.

[0125] A penetrating injury to the wall of the eye disrupts the continuity of the sclera, and more importantly, can compromise the internal pressure of the eye. This can cause the internal contents of the eye, e.g. the vitreous humor, to release from the eye, and can potentially lead to prolapse of the spherical structure. The thin layer of retinal tissue on the posterior wall may detach as a result of the drop in pressure and loss of shape. Sealing the penetrating injury stops further release of vitreous humor and allows the aqueous humor production to re-establish internal pressure. In one or more preferred embodiments, a method of administering a hydrogel is provided in which the penetrating injury is completely sealed around its margins, whether the margins are regular or irregular.

[0126] In some embodiments, a hydrogel patch is provided in a fluid, unsupported form. For example, the hydrogel may be injected from a suitable device through the site of the penetrating injury. The injection may be conducted to allow a bolus of hydrogel material to deposit in the interior chamber of the eye. Preferably, the internal bolus has a perimeter larger than the perimeter of the margins of the injury, to thereby allow proper coverage and sealing. Delivery of the hydrogel may then continue through the margin plane. Delivery of the hydrogel continues over the exterior of the plane of the injury, e.g., to thereby form an exterior cap over the injury. Generally, the hydrogel is then allowed time to cure. Thermal energy may be applied, for example in the form of a heat lamp, to help accelerate the fixation. In some embodiments, the hydrogel forms a rivet like structure which improves its ability to seal the penetration as the internal pressure in the eye builds. At an appropriate time, the hydrogel plug may be removed, e.g., by applying cold water. Upon application of cold water, the polymer is rehydrated to its fluid form to thereby facilitate its removal.

[0127] Generally, the hydrogel is used for temporary occlusion/sealing of ocular perforations. Illustrative temporary time periods include periods of less than about 30 days. For example, the hydrogel plug may be removed within about 30 days following administration, or within about 20 days following administration, or within about 15 days following administration. Typically, the hydrogel plug will remain in its deployed position for no more than about 7-10 days.

[0128] Unlike other adhesives used in the body (e.g., cyanoacrylate, fibrin glue), the adhesive nature of the PNIPAM-copolymer based hydrogel is easily reversed by simply lowering the temperature. Therefore, closure of ocular perforations can be performed temporarily, then reopened easily. This is convenient in cases where multi-stage surgeries may be required to repair the eye. Rather than place sutures in the eye between procedures—a process which can cause additional damage and create discomfort for the patient—this temporary adhesive allows easy re-entry.

[0129] Advantages of the instant compositions, methods and devices provided herein include the ability to close an ocular perforation while maintaining a low profile on the eye surface, thereby eliminating frictional discomfort. Another significant advantage is the reversibility of adhesion. For example, in combat medicine, casualties presenting with ocular trauma are typically first stabilized at surgical hospitals (FSH) or combat service hospitals (CSH), until they can be air lifted to base hospitals with better equipment and more specialized medical personnel that can perform more elaborate procedures. In the event of ocular trauma in the battlefield, these casualties' eyes may be debrided and sealed to prevent complete loss of the eye, but reattachment of retina or other reconstructive procedures may be postponed until transport to base hospital facilities. Additional trauma from suture placement may be avoided by temporarily sealing perforations using the hydrogels and related methods described herein, followed by removing the hydrogel by lowering its temperature and allowing it to release.

EXAMPLES

[0130] The following examples are put forth to provide those of ordinary skill in the art with a complete disclosure and description of how the compositions, hydrogels, and methods provided herein are made and evaluated, and are intended to be purely exemplary. Thus, the examples are in no way intended to limit the scope of what the inventors regard as their invention. There are numerous variations and combinations of reaction conditions, e. g., component concentrations, desired solvents, solvent mixtures, temperatures, pressures, and other reaction parameters and conditions that may be employed to optimize product characteristics such as stability, purity, mechanical properties, yield, and the like. Such are considered as well within the scope of the present disclosure.

Example 1

PNIPAM Copolymer Synthesis

[0131] Copolymer of NIPAM and N-tert-but lacrnicle $N_{85}T_{15}$): NIPAAM (4.75 g), N-tert-butylacrylamide (0.75 g) and 2,2'azobisisobutyronitrile (AIBN, 0.021 g) were dissolved in a mixture of 37.5 ml of dry tetrahydrofuran and 12.5 ml of benzene. The magnetically stirred solution was degassed, heated to 50° C. for 24 hours under positive nitrogen pressure, and allowed to cool. The reaction mixture was filtered (0.45μ Teflon filter) and the filtrate volume reduced by half. Ether was added with mixing to precipitate the copolymer. The precipitate was filtered off, washed with ether, and dried under vacuum to yield dry 4.64 gram of copolymer product.

[0132] Copolymer molar mass moment and polydispersity were characterized:

TABLE 3

Molar mass moments (g/mol)	Polydispersity
M_n 6.624×10^5 (±1.506%) M_p 4.775×10^5 (±1.025%) M_w 7.143×10^5 (±1.954%) M_z 7.862×10^5 (±5.333%)	M_w/M_n 1.078 (±2.467%) M_z/M_n 1.187 (±5.541%)

[0133] In the next step, 10%, 15%, 20% and 30% solutions were prepared by dissolving the copolymer in DI water by using a horn sonicator to provide representative hydrogels.

[0134] Copolymer of NIPAM and Butylacrylate: This copolymer was obtained from Sigma Aldrich (molecular weight: M_n 30,000). Aqueous mixtures: 10%, 15%, 20% and 30% weight percent solids were prepared.

[0135] The following table provides a summary of exemplary hydrogels that were evaluated.

TABLE 4

	PNIPAM	PNIPAM:N-tert Butylacrylamide (A_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)
Average 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 (° C.):	32	25	14-16

Example 2

Adhesion Evaluation

[0136] Free-standing pNIPAM-based gels as described in Example 1 were synthesized using a wet chemistry approach, characterized and stored at low temperature prior to use. Adhesion to dissected cadaveric porcine scleral tissue was characterized using a uniaxial tension tester to test under ideal normal force conditions.

[0137] An in vitro cadaveric porcine eye model was utilized to assess the ability of the gels to seal penetrating incisions through the sclera, mimicking clinical cases. (Kaja, et al., *Clinical Ophthalmology* (Auckland, NA), 2012; 6:305-309; Lopez-Guajardo, L., et al., *Invest. Ophthalmol Vis Sci.* 2011; June 8; 52(7):4080-4). Adhesion in each test was compared against medical-grade cyanoacrylate glue and sutures, respectively. Real-time IOP (intraocular pressure) was tracked in the whole porcine eye using 19-gauge catheter pressure transducer inserted through the pars plana.

[0138] The studies were conducted to assess one or more of the following: whether the pNIPAM-copolymer gels are capable of (i) meeting the adhesion performance of cyanoacrylate in uniaxial tension testing, (ii) preventing hypotony in a cadaveric porcine eye, and/or maintain IOP comparable to suture. Additionally, the copolymers were assessed to determine whether hydrogels as provided herein comprising the pNIPAM-based copolymers can be removed using a temperature lowering protocol.

[0139] Using a modified syringe device designed to administer the hydrogels provided herein, the co-polymer was carefully injected into the posterior chamber of the eye near the perforation, and, while continuously deploying the co-polymer, the syringe was slowly retracted from the perforation, leaving behind a trail of co-polymer through the perforation tract. At the exterior surface of the sclera, additional co-polymer was deposited, creating a mushroom like "cap" on the ocular surface. The polymer was allowed to settle for several seconds, enabling it to heat up and dehydrate. After, a scalpel was used to carefully cut away the surface cap. The table below provides a summary of PNIPAM-copolymers/hydrogels evaluated, the ocular pressures maintained, and whether or not they passed the in vitro IOP test. A series of PNIPAM co-polymer preparations were tested in this manner and were given a pass if they were able to maintain ocular pressures above 70 mm Hg (an arbitrary performance criterion). At least five different preparations met the foregoing criterion, and an additional two preparations were able to maintain pressures up to 40 mm Hg, or twice a clinically high IOP level. As can be seen, while all of the PNIPAM compositions failed to maintain an ocular pressure, several of the illustrative copolymer-based hydrogels were able to maintain a measurable ocular pressure.

TABLE 5

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

[0140] Results: Synthesized pNIPAM-based copolymer comprising gels predictably and reversibly transitioned between adhesive and non-adhesive states in the desired temperature range for scleral closure. Uniaxial tension testing yielded adhesion performance data comparable to cyanoacrylate with some gel formulations. Intraocular pressure results from the porcine eye model showed that IOP as high as 70-77 mm Hg could be maintained for sustained periods without any leakage. Performance in both tests varied as a function of placement procedure, chemical formula, molecular weight, and gel solution concentration. Gel detachment was successfully achieved by irrigation of the placement site with cold water.

[0141] Thus, the instant pNIPAM-based gel adhesives can be effective to provide a rapid and reversible approach for temporarily and satisfactorily sealing scleral penetrations. Such adhesives can provide a new reversible technique for temporary intervention in ocular trauma and other applications.

Example 3

Evaluation of IOP in Rabbits

[0142] Baseline was established by measuring IOP on both eyes of two rabbits twice a day (AM and PM) for ten days. IOP was measured using an iCare® Tonovet with a magnetically actuated tonometer. The Tonovet calculates an average reading from six tonometric measurements taken in succession. Four successive readings were taken on each eye, thus 24 measures contributed to the averaged IOP for each eye. For all tonometry measures rabbits were removed from the cage and placed on an evaluation table for 2 minutes to allow the animal to relax. The average IOP results show no significant difference between right and left eyes, and it also shows no significant difference between morning and afternoon average IOP. These results suggested that using an untreated eye as a control to compare against the treated eye in the same animal was a valid assumption.

[0143] Implantation Procedure. The first two rabbits underwent surgical procedures to create a 3 mm penetrating injury of the sclera, in the right eye (OD). A 3 mm penetrating incision was created in the sclera, temporal and superior to the cornea, approximately 3 mm from the limbus with the axis of the cut following the perimeter of the limbus. Rabbit 01 (No.116) was randomized to the adhesive group. Rabbit 02 (No. 117) was randomized to the control (no treatment) group. All surgical procedures performed on these animals were in compliance with IACUC guidance and requirements.

[0144] First a 3 mm incision was created and confirmed using calipers. B) Next the hydrogel as described in Example 1 (PNIPAM-butylacrylate copolymer) was deployed using a refrigerated and sterile 1 cc syringe. C) Once deployed, the translucent hydrogel was seen occluding the incision. D) As the hydrogel began to heat up from the inside, the internal portion was seen to transition to opaque. E) As the temperature of the entire hydrogel rose, the rest of the hydrogel transitioned to a white opaque appearance. F) After 5 minutes, the hydrogel was completely opaque white and small droplets of water precipitating from inside the hydrogel were seen on the surface. G) Once set, the "cap" of the hydrogel was clipped using surgical shears, leaving behind H) a small "plug". I) Lastly, the conjunctiva was pulled over the hydrogel plug; no sutures were placed.

[0145] A similar 3 mm incision was created in the right eye of rabbit (R02), but no treatment was administered. The eye in R02 was left to heal on its own. IOP was measured on all four eyes in the late afternoon on the same day, and subsequently measured once in the morning and in the afternoon for 5-days.

[0146] Results: The hydrogel-treated eye maintained higher IOP than the untreated eye for 72 hours. IOP measurements of both eyes for each rabbit (R01 and R02) were measured once in the morning and afternoon for 72-hours following the morning implantation procedure. The IOP measurements of each traumatized eye was normalized against the IOP of the opposite (control) eye in the same rabbit, and plotted in FIG. **5**. Over the 72-hour period, the treated eye showed approximately 20%-30% higher IOP vs. the untreated eye, when each was normalized against the contralateral eye (control). The absolute IOP of the traumatized eye without treatment consistently measured 2 mm Hg over the 72-hour period.

[0147] At day-5, both rabbits were sedated to evaluate status of incision and to confirm if the hydrogel had migrated or remained in place. Upon inspection, it was determined that the hydrogel was intact. No visible indications of infection or irritation were visible at the immediate site after 5 days.

Example 4

Sterile Hydrogel Deployment in Rabbits Using Exemplary Deployment Device

[0148] A method was developed for preparation of a sterile hydrogel comprising a PNIPAM copolymer which can be implanted for in vivo characterization. Pigmented New Zealand rabbits (~2 kg) were randomized to either treatment group (receiving hydrogel) or control (no treatment). Baseline IOP was measured for both eyes (OD and OS) of all animals over a three day period prior to implantation using a Tonovet® rebound tonometer (using canine setting).

[0149] Surgical Procedure. 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 3 mm 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 group OD eyes were then treated with hydrogel.

[0150] Hydrogel deployment was performed using a modified, sterile 1 cc syringe. Approximately 0.3 cc to 0.4 cc of sterile hydrogel was extracted from a crimp top vial using the syringe (no needle) with care not to aspirate bubbles into the chamber. Excess hydrogel was wiped away from the tip of the syringe using sterile gauze. The syringe was then placed inside an autoclave-sterilized customized 20 mL syringe. The volume created between the 20 mL syringe and the 1 cc syringe was subsequently filled with a mixture of ammonium nitrate and water to induce an endothermic chemical reaction to cool the hydrogel during deployment. 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 1 cc syringe and the hydrogel was deployed on the eye.

[0151] The catheter tip of the injector tool was inserted into the 3 mm 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 0.3 cc of hydrogel was used for all eyes. An incandescent lamp was positioned near the eye so that the hydrogel surface temperature was held at 32.5 C 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.

[0152] Post-Procedure Monitoring. Animals were checked regularly for signs of infection, discomfort or other adverse effects. Pain medication (ketofen 3 mg/kg) was administered for 48 hrs PRN. IOP was monitored in both eyes of each animal at least twice daily following the surgical procedure. IOP_{OD} for each measurement point was normalized vs the contralateral eye of the same animal (IOP_{OS}), to normalize for any effects that may have been caused by stress or medications.

[0153] Once water was introduced into the endothermic reaction chamber of the injector tool, time recording of the procedure was initiated. Two minutes were elapsed to allow the reactants to mix. Average surface temperature readings of the injector tool were 9° C., well below the LCST for this hydrogel formulation (LCST=14-16 C).

[0154] After two minutes, hydrogel deployment was initiated. In all instances when the injector tool's surface temperature was T=9° C., the hydrogel deployed smoothly and easily. After only 30 seconds deployment the hydrogel began to transition to a white opaque color, indicative of its rise above the its lower critical solution temperature (T) and subsequent dehydration. After five minutes, the gel was completely opaque and beads of water were seen on the surface. After five minutes, using surgical scissors, the excess hydrogel "cap" was trimmed away from the surface to create a low profile "flathead" and the conjunctiva was gently drawn over the hydrogel. The average time to deploy the hydrogel in the first series of cases was less than nine minutes (n=7). Based upon these results, it was determined that the instant PNIPAM copolymer-based hydrogels, once deployed to an ocular trauma site, can seal 3 mm penetrating injuries within ten minutes from procedure initiation.

[0155] Penetration of the scleral surface with the micro vitreoretinal blade caused an immediate drop in IOP, resulting from the scleral wall being compromised. Eyes sealed by the hydrogel exhibited a refractory period of between 12 to 24 hrs following the procedure, during which the ciliary epithelium of the eye produced aqueous humor to reestablish normal IOP. This was consistent with known rates of aqueous humor production from the eye. In sum, all treatment eyes (i.e. those that received the hydrogel) underwent an approximate 12 hr-24 hr refractory period of low IOP (IOP_{OD}=2 mm Hg).

[0156] During the procedures, it was observed that preferred deployment of the unsupported hydrogel (i.e., not deposited on a substrate) comprises creation of a "rivet like" structure where a spherical cap of hydrogel is created on the interior surface of the scleral, with hydrogel filling through the perforation, followed by a cap.

[0157] Scleral tissue surround the implant sites show no signs of redness, inflammation or bleeding after 48 hrs, suggesting that the hydrogel induces no adverse tissue response.

[0158] The components, steps, features, objects, benefits and advantages which have been discussed are merely illustrative. None of them, nor the discussions relating to them, are intended to limit the scope of protection in any way. Numerous other embodiments are also contemplated. These include embodiments which have fewer, additional, and/or different components, steps, features, objects, benefits and advantages. These also include embodiments in which the components and/or steps are arranged and/or ordered differently.

[0159] Unless otherwise stated, all measurements, values, ratings, positions, magnitudes, sizes, and other specifications which are set forth in this specification are approximate, not exact. They are intended to have a reasonable range which is consistent with the functions to which they relate and with what is customary in the art to which they pertain.

[0160] All articles, patents, patent applications, and other publications which have been cited are hereby incorporated herein by reference.

1. A temperature-responsive hydrogel comprising a poly (N-isopropylacrylamide) copolymer at a concentration of about 10 weight percent to about 60 weight percent in water, wherein the copolymer (i) is a copolymer of poly(N-isopropylacrylamide) and a second polymer that is either N-tert-butylacrylamide or butylacrylate, (ii) has a weight percent ratio of poly(N-isopropylacrylamide) to the second polymer of about 99:1 to about 50:50, and (iii) has a number average molecular weight of about 5,000 to about 5,000,000 daltons.

2. The hydrogel of claim **1**, wherein the poly(N-isopropylacrylamide) copolymer is a poly(N-isopropylacrylamide): N-tert-butylacrylamide copolymer.

3. The hydrogel of claim **1**, wherein the poly(N-isopropylacrylamide) copolymer is a poly(N-isopropylacrylamide): butylacrylate copolymer.

4. The hydrogel of claim **1**, wherein the weight percent ratio of poly(N-isopropylacrylamide) to the second polymer is selected from 99:1, 95:5, 90:10, 85:15, 80:20, 75:25, 70:30, 65:35, 60:40, 55:45 and 50:50.

5. The hydrogel of claim **1**, wherein the weight percent ratio of poly(N-isopropylacrylamide) to the second polymer is in a range of about 95:5 to about 70:30.

6. The hydrogel of claim **1**, wherein the copolymer has a number average molecular weight of about 10,000 to about 3,000,000 daltons.

7. The hydrogel of claim **1**, wherein the copolymer has a number average molecular weight of about 20,000 to about 2,000,000 daltons.

8. The hydrogel of claim **1**, having a copolymer concentration in water selected from the group consisting of about 10 weight percent, 15 weight percent, 20 weight percent, 25 weight percent 30 weight percent, 35 weight percent, 40 weight percent, 45 weight percent, 50 weight percent, 55 weight percent, and 60 weight percent in water.

9. The hydrogel of claim **1**, having a lower critical solution temperature in a range of about 10° C. to about 35° C.

10. The hydrogel of claim **1**, wherein the poly(N-isopropylacrylamide) copolymer is a block copolymer.

11. The hydrogel of claim **1**, further comprising an excipient or additive.

12. The hydrogel of claim **1** in sterile form.

13. The hydrogel of claim **1**, having an adhesive strength of in a range between 10 mN to 10,000 mN when measured using an in vitro uniaxial adhesion test to scleral tissue at 37° C.

14. The hydrogel of claim **1** further comprising a bioactive agent.

15. An adhesive patch comprising the hydrogel of claim **1**.

16. The adhesive patch of claim **15**, comprising the hydrogel deposited on a polymeric substrate.

17. The adhesive patch of claim **16**, wherein the polymeric substrate is selected from the group consisting of parylene, poly-lactic acid, polyimide, and polydimethylsiloxane.

18. A method for reversibly sealing an ocular perforation, the method comprising applying a hydrogel of claim **1** to a tear in ocular tissue of a subject in an amount effective to seal the tear, wherein when exposed to a temperature above its critical solution temperature, the hydrogel becomes adhesive, and when exposed to a temperature below its critical solution temperature, the hydrogel becomes less adhesive.

19. The method of claim **18**, wherein the hydrogel is maintained at a temperature below its critical solution temperature prior to said applying.

20. The method of claim **18**, wherein the temperature of the ocular tissue is above the critical solution temperature of the hydrogel.

21. The method of claim **20**, wherein the hydrogel adheres to the tissue of the edges of the tear.

22. The method of claim **18**, wherein said applying comprises applying to an inner surface of the eye wall a slight excess of an amount of hydrogel effective to fill a void created by the ocular tear.

23. The method of claim **22**, wherein the ocular pressure is effective to press the excess hydrogel against the inner surface of the eye to thereby create an internal ocular seal.

24. A device for delivery of a temperature responsive hydrogel, the device comprising (i) a first chamber for containing a temperature-responsive hydrogel, (ii) a second chamber at least partially surrounding the first chamber, said second chamber capable of maintaining a particular temperature or temperature range before and/or during delivery of the hydrogel, (iii) a port for delivery of the hydrogel from the first chamber to the delivery site, and (iv) a mechanism for delivery of the hydrogel from the first chamber to the delivery site.

25. The device of claim **24**, wherein the second chamber includes a cooling mechanism or material.

26. The device of claim **25**, wherein the first chamber has a volume of 0.5 mL to 5 mL.

27. The device of claim **25**, wherein the size of the second chamber relative to the first chamber ranges from about 50:1 to about 10:1.

28. The device of claim **24**, wherein said second chamber further comprises a port for introduction of a coolant material.

29. The device of claim **24**, comprising in the first chamber the hydrogel of claim

30. The device of claim **29**, comprising in the second chamber one or more materials effective to carry out an endothermic reaction.

31. The device of claim **30**, wherein said one or more materials are sequestered from one another prior to reaction.

32. The device of claim **30**, wherein said second chamber comprises ammonium nitrate.

33. The device of claim **24**, further comprising one or more tools effective to facilitate removal of a hydrogel plug from the eye.

34. The device of claim **24**, further comprising means for aspiration or irrigation of ocular tissue.

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