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Award Number: **W81XWH-12-1-0314**

TITLE: **VRPI Thermoresponsive Reversibly Attachable Patch for Temporary Intervention in Ocular Trauma**

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REPORT DATE: September 2014

TYPE OF REPORT: **Annual Report**

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

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1. REPORT DATE September 2014		2. REPORT TYPE Annual Report		3. DATES COVERED 15-AUG-2013 to 14-AUG-2014	
4. TITLE AND SUBTITLE VRPI Thermoresponse Reversibly Attachable Patch for Temporary Intervention in Ocular Trauma				5a. CONTRACT NUMBER	
				5b. GRANT NUMBER W81XWH-12-1-0314	
				5c. PROGRAM ELEMENT NUMBER	
6. AUTHOR(S) Mark S. Humayun E-Mail: humayun@usc.edu				5d. PROJECT NUMBER	
				5e. TASK NUMBER	
				5f. WORK UNIT NUMBER	
7. PERFORMING ORGANIZATION NAME(S) AND ADDRESS(ES) University of Southern California Los Angeles CA 90089				8. PERFORMING ORGANIZATION REPORT NUMBER	
9. SPONSORING / MONITORING AGENCY NAME(S) AND ADDRESS(ES) U.S. Army Medical Research and Materiel Command Fort Detrick, Maryland 21702-5012				10. SPONSOR/MONITOR'S ACRONYM(S)	
				11. SPONSOR/MONITOR'S REPORT NUMBER(S)	
12. DISTRIBUTION / AVAILABILITY STATEMENT Approved for Public Release; Distribution Unlimited					
13. SUPPLEMENTARY NOTES					
14. ABSTRACT Penetrating injuries to the eye can lead to drops in intraocular pressure and subsequent retinal detachment and loss of vision, if not managed properly. The current standard of care to close scleratomies and other perforations of the sclera are to place sutures which are uncomfortable and can lead to abrasion and infection from eye rubbing. Glues are currently not approved in the US for closure of scleral tears. Here we fabricate and test, both in vitro and in vivo, sutureless wound closure patches for the eye. The enabling technology is a thermo-reversible adhesive (poly n-isopropyl acrylamide), pNIPAM, which is adhesive to tissues at body temperature and non-adhesive at room temperature. Here we prepare a series of different pNIPAM scleral patches and test two key properties in vitro: 1) ability to survive ETO sterilization and extreme temperature, and adhesion strength to scleral tissue both in a uniaxial pull test and in an in vitro, porcine tissue eye model. Results are compared against					
15. SUBJECT TERMS Nothing Listed					
16. SECURITY CLASSIFICATION OF:			17. LIMITATION OF ABSTRACT	18. NUMBER OF PAGES	19a. NAME OF RESPONSIBLE PERSON USAMRMC
a. REPORT U	b. ABSTRACT U	c. THIS PAGE U			19b. TELEPHONE NUMBER (include area code)
			UU	22	

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1. Introduction.

Scleral penetrating trauma causes immediate loss of intraocular pressure (IOP) when the eye wall is penetrated, for example, by a foreign body. Sustained loss of IOP (hypotony) for greater than 24 hours can lead to retinal detachment and subsequent permanent vision loss, as the retina is metabolically supported by the choroidal vasculature attached to the inner surface of the sclera. Therefore, there is a strong motivation to develop rapidly deployable temporary interventions to treat scleral penetrating injuries. The purpose of this project is to evaluate a novel, thermo-responsive, reversibly attachable hydrogel to seal penetrating injuries at or near the time of injury to provide temporary intervention. The scope of this project is to fabricate adhesive hydrogels, characterize their adhesive properties in vitro, validate adhesive performance in vivo and perform preliminary biocompatibility assessments.

2. Keywords.

sutureless wound repair, sclera, hydrogel, PNIPAM, ocular trauma, scleral penetrating injury, intraocular pressure, reversible adhesive, biomaterials, biocompatibility

3. Overall Project Summary.

The following tasks highlighted in bold were identified as deliverables for year two of this program:

<u>Milestone</u>	<u>Completion</u>
Fabricate test patches	Q 1
Sterilize patches	Q 1
Temperature exposure of patches	Q 1
Adhesion performance characterization	Q 3
Time to attach/detach test	Q 4
Fabricate test patches	Q 5
Identify best patches & fabricate more	Q 5
In vivo sclerotomy closure test	Q 6
In vivo peritomy test	Q 7
Histology	Q 7
Review all data & summary report	Q 8

3.1 Test Patch Fabrication and Preliminary Characterization.

3.1.1 Background.

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

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

3.1.2 Objectives.

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

3.1.3 Methods.

Hydrogel Synthesis. Hydrogel co-polymer chemistries were prepared via ATRP synthesis technique using combinations of NIPAM monomer with either n-tert butylacrylamide or butylacrylate. **Table 3.1** shows the chemistries that were prepared along with additional characteristics and properties.

Table 3.1.1 Co-Polymers Tested			
	PINPAM	PNIPAM:N-tert Butylacrylamide (N _x T _y)	PNIPAM:Butylacrylate (N _x BA _y)
Chemical Formulae:	(C ₆ H ₁₁ NO) _x	(C ₆ H ₁₁ NO) _x :(C ₇ H ₁₃ NO) _y	(C ₆ H ₁₁ NO) _x :(C ₇ H ₁₂ O ₂) _y
Co-Polymer Ratios Tested:	N/A	(85:15)	(95:5); (88:12)
Avg. Molecular Weights:	2.864 x 10 ⁵	5.55 x 10 ⁵ to 6.624 x 10 ⁵	3.00 x 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

IOP Testing Setup Procedure. Whole porcine eyes (cadaveric) were used to assess adhesion and ability to maintain IOP. Fresh (harvested within 24hrs) porcine eyes (Sierra for Medical Sciences, Whittier CA) were mounted into a styrofoam fixture and immobilized with dissection pins. Partial vitrectomies were performed on each eye (Alcon, Constellation). IOP was controlled using an electronically controlled saline infusion coupled with a digital pressure sensor, **Figure 3.1.1**.

A single 3mm incision was created in the sclera, approximately 3mm distance radial from the limbus, with the incision path running tangential to the limbus perimeter. Heated saline infusion leaking from the incision was used to confirm penetration through the scleral tissue. Infusion was then shut off and the surface dried to enable placement of the hydrogel.

Hydrogel was deployed at the incision creating a rivet-like plug of hydrogel through the incision tract. After placement, an incandescent lamp was used to locally heat the surface temperature of the hydrogel to 32–33C for 5 minutes to set the hydrogel. Afterwards, excess hydrogel was trimmed away.

IOP Testing. Once the hydrogel was set, the IOP was slowly raised from baseline by manually increasing the saline infusion pump pressure until leakage was observed or the pressure sensor value no longer increased with increasing infusion rate (indicative of a non-visible leak). The maximum pressure (mm Hg) was recorded as maximum pressure held.

3.1.4 Results and Discussion.

Figure 3.1.2 is a representative photograph of a porcine eye that has been properly fixed with hydrogel (red arrow). In these instances the hydrogel formed a small rivet-head on the interior surface of the eye, which assisted with sealing the incision, when infusion was applied.

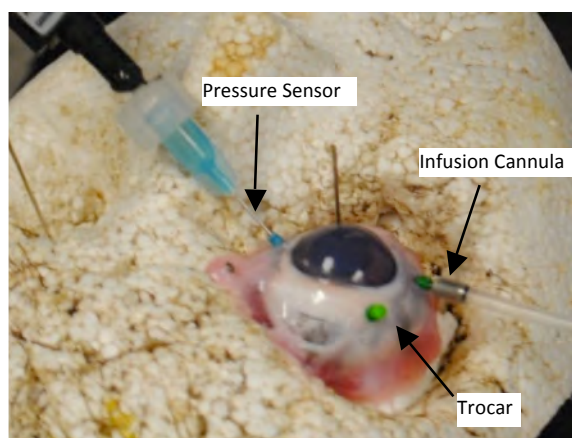


Fig 3.1.1. In Vitro IOP test system using whole porcine eye (cadaveric) with heated saline infusion and digital pressure sensor.

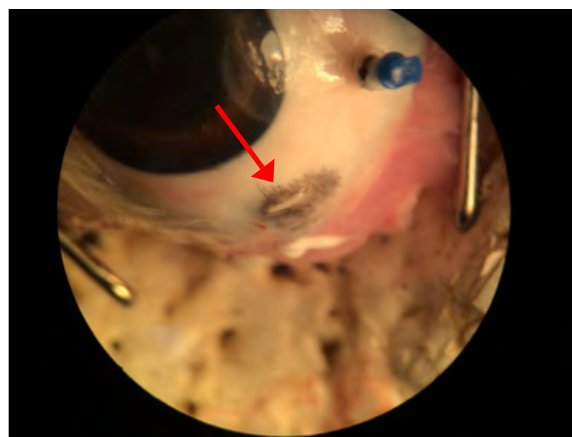


Fig 3.1.2 Optical micrograph of porcine eye with 3mm incision which has been sealed with hydrogel and maintained pressure after infusion with saline.

A. Both co-polymer compositions improved adhesive performance of PNIPAM hydrogel. Table 3.1.2 lists all of the different chemistry formulations tested. Tests evaluating PNIPAM co-polymerized with butylacrylate showed consistently higher pressure values with most IOP measured at 77mm Hg. It was decided that further efforts would focus on PNIPAM-butylacrylate compositions.

B. The porcine eye model shows some inconsistencies in data recording. Repeated testing using the whole eye IOP test model showed wide ranges in variation. Differences in the amount of vitreous removed, and the location of the vitreous with respect to the rest of the test setup (e.g. pressure sensor location, infusion cannula location) led to wide variance in observed IOP. It was decided that a more controllable IOP test system should be designed to reduce variations from test to test.

Table 3.1.2. IOP Adhesion Performance Data for Different Hydrogel Chemistries							
Date	Compound	Co-P Ratio	LCST	MW (Avg)	% [Aqueous]	Maximum Pressure Held (mmHg)	Pass/Fail
7.15.2013	PNIPAM	N/A	32	2.864×10 ⁵ (±2.474%)	0.8	0	F
7.15.2013	PNIPAM	N/A	32	2.864×10 ⁵ (±2.474%)	2	0	F
7.15.2013	PNIPAM	N/A	32	2.864×10 ⁵ (±2.474%)	5.26	0	F
9.25.2013	PNIPAM	N/A	32	10,000	10.0	0	F
9.25.2013	PNIPAM	N/A	32	10,000	14.2	0	F
9.25.2013	PNIPAM	N/A	32	10,000	25.0	0	F
9.25.2013	PNIPAM	N/A	32	10,000	30.0	0	F
9.25.2013	PNIPAM	N/A	32	10,000	43.2	0	F
11.22.2013	PNIPAM:n-tert	85:15	25	1.038×10 ⁶ (±2.583%)	No	No	No
12.6.2013	PNIPAM:n-tert	85:15	25	6.624×10 ⁵ (±1.506%)	10.0	0	F
12.6.2013	PNIPAM:n-tert	85:15	25	6.624×10 ⁵ (±1.506%)	15.0	0	F
12.6.2013	PNIPAM:n-tert	85:15	25	6.624×10 ⁵ (±1.506%)	20.0	40	F
12.6.2013	PNIPAM:n-tert	85:15	25	6.624×10 ⁵ (±1.506%)	30.0	77	P
10.31.2013	PNIPAM:n-tert	85:15	25	5.55×10 ⁵ (±1.472%)	10.0	0-10	F
10.31.2013	PNIPAM:butylacrylate	95:5	25	3×10 ⁴	10.0	0	F
10.31.2013	PNIPAM:butylacrylate	95:5	25	3×10 ⁴	15.0	77.4	P
10.31.2013	PNIPAM:butylacrylate	95:5	25	3×10 ⁴	20.0	77.2	P
10.31.2013	PNIPAM:butylacrylate	95:5	25	3×10 ⁴	30.0	77.9	P
11.13.2013	PNIPAM:butylacrylate	88:12	14-16	3×10 ⁴	10.0	0	F
11.13.2013	PNIPAM:butylacrylate	88:12	14-16	3×10 ⁴	15.0	40	F
11.13.2013	PNIPAM:butylacrylate	88:12	14-16	3×10 ⁴	20.0	77.2	P
11.13.2013	PNIPAM:butylacrylate	88:12	14-16	3×10 ⁴	30.0	N/A (too viscous)	F

3.2 Characterizing Hydrogel Adhesion - Development of New IOP Test System

3.2.1 Background.

IOP testing using whole porcine eyes that have undergone vitrectomy and cannulated with saline infusion and a digital pressure sensor showed wide variations in recorded IOP measurements under relatively similar test conditions. There is a strong motivation to develop a more easily controlled IOP test system which enables consistent creation of 3mm (or larger) scleral penetrations on cadaveric scleral tissue, and also allows for more consistently and accurately controlled internal pressure (IOP).

3.2.2 Objectives.

- Setup cadaveric porcine eye IOP Testing System and validate.
- Perform IOP Testing to quantify adhesion strength of different unsupported and supported hydrogel adhesives.
- Determine which formulations exhibit best performance.

3.2.3 Methods.

Original IOP Testing System. Preliminary IOP measurements using cadaveric porcine eyes yielded wide variations in results when testing the same samples. Variations in the vitrectomy performed to prepare each eye, as well as, variations in freshness of tissue resulted in wide variations in measured IOP.

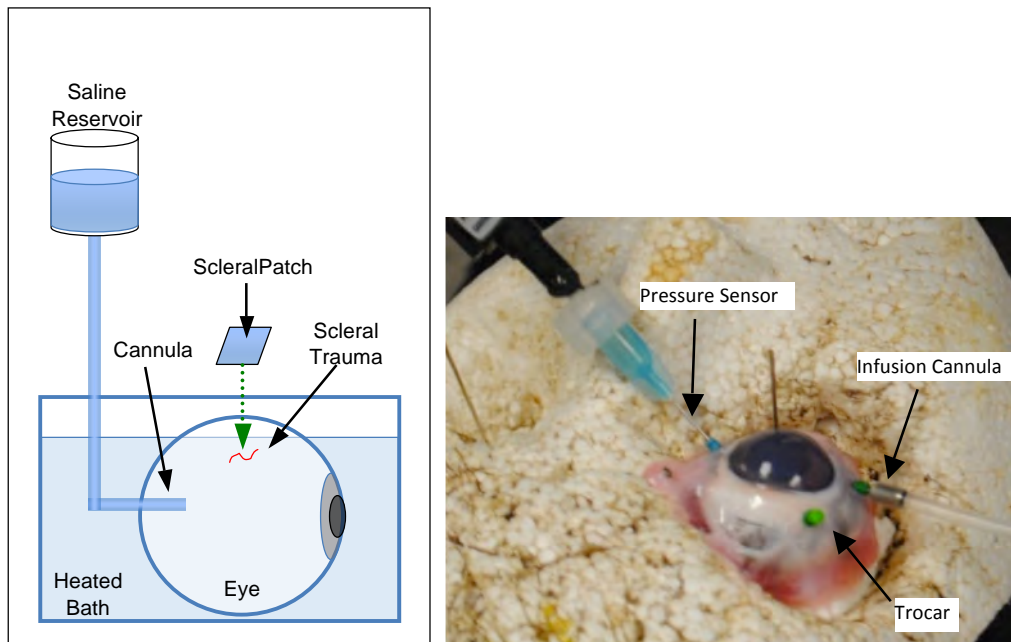


Fig 3.2.1. Schematic (left) and photograph (right) of in vitro IOP test system using whole porcine eye (cadaveric) with heated saline infusion and digital pressure sensor.

Revised IOP Testing System. A revised IOP testing system was designed that would allow us to repeatedly create similar penetrating injuries in scleral tissue, but which would also allow us to very carefully regulate the intraocular pressure to which the test specimen was subjected. **Figure 3.2.2** (next page) shows the design of the revised IOP Test system. Porcine sclera was dissected from the eyes used in the previous testing system, and mounted (a) in a modified 60mL syringe (b). The syringe tip was machined to create an 8 mm diameter aperture where the scleral tissue was positioned. A modified plunger was created to fix the scleral tissue in place. The normal plunger (d) was then inserted behind the modified plunger. A small port was created on the sidewall of the syringe (c) to both load the syringe with heated phosphate buffered saline and to insert a digital pressure sensor to track IOP. IOP was controlled by connecting the syringe to a digital and automated infusion system (yellow device lower left), which allows pressure to be applied to the syringe plunger at a carefully controlled rate. This system allowed pieces of scleral tissue with varying sized/design penetrating injuries to be tested.

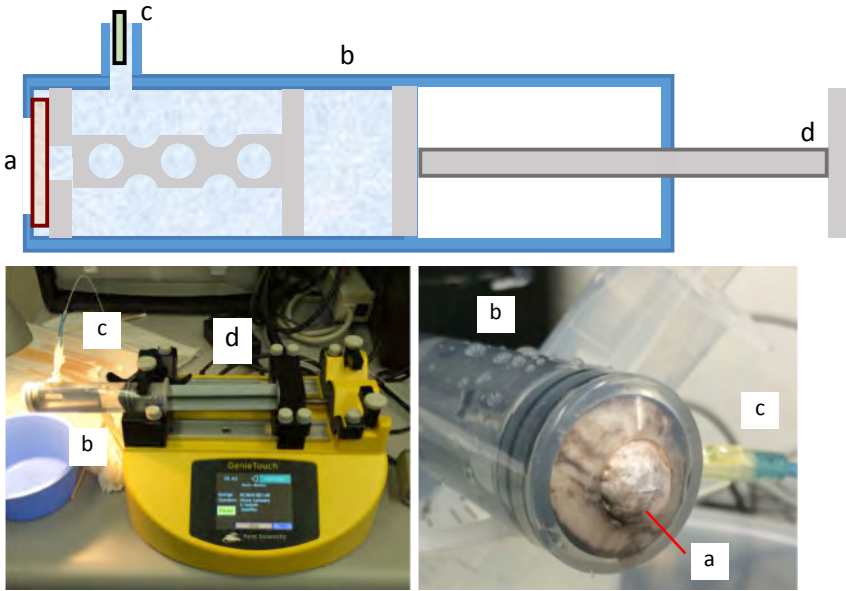


Fig 3.2.2. (top) Cross-sectional schematic and associated photographs (bottom) of revised IOP test system in which hydrogel samples are tested on a dissected section of scleral tissue. Dissected sclera(a) is mounted into a modified 60mL syringe (b) with a custom port (c) for filling with saline and insertion of the pressure sensor. Pressure is controlled using a digitally controlled infusion system connected to the syringe

3.2.4 Results and Discussion.

A. Mechanical eye IOP test system yields more consistent test measurement data. 0.3 mL of 30% hydrogel of 95% PNIPAM:5% butylacrylate was mounted onto the test system and allowed to set at 32.5C for 15 minutes prior to the start of each test trial.

Figure 3.2.3 (next page) shows preliminary results from IOP testing unsupported hydrogel adhesive with the new system. All three measurements were taken using the same quantities (0.3mL) of the same hydrogel chemistry on three different dissected pieces of sclera. Time to set, and surface temperature were both carefully controlled for all three tests. While there is some variation in the measurements, the majority of the tests performed are showing significantly more consistency. Averaged data on each test specimen will be reported in future quarters.

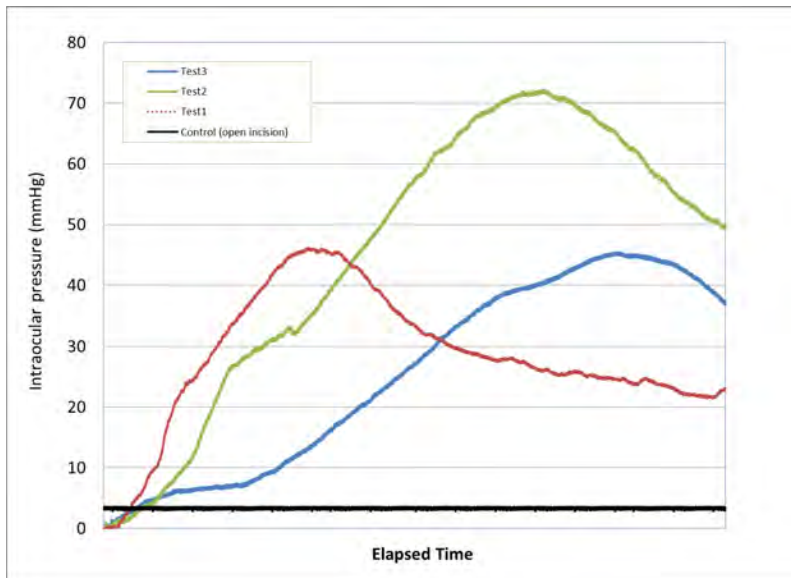


Fig 3.2.3. IOP vs. time recordings recorded using the new IOP test system. All three scans recorded from three hydrogel test runs using the same formulation. Pressure recordings for an unsealed scleral tissue incision was run (black) to show baseline pressure.

3.3 In vivo Sclerotomy Closure

3.3.1 Background.

Copolymer hydrogels of 95% N-isopropylacrylamide (NIPAM): 5% butylacrylate with a hydration percentage of 70% (i.e. 30 weight% copolymer) exhibited the highest tensile adhesion strength. The goal of this part of the project was to perform repeated in vitro IOP simulation testing to quantify adhesion performance in a more appropriate testing model.

3.3.2 Objectives.

- Compare IOP of eyes sustaining 3mm scleral penetrating injury treated with hydrogel adhesive vs. IOP of eyes sustaining 3mm scleral penetration receiving no treatment (control).
- IOP data on traumatized eyes reported as normalized vs. contralesional eye of same animal.

3.3.3 Methods.

Preparation of Sterile Hydrogel. 0.857 grams of poly(NIPAM-cobutylacrylate) hydrogel powder was loaded into a glass crimp-top vial, which was then placed in a sterilization pouch with vial cap and ethylene oxide sterilized. After sterilization, the top was placed on the vial and crimped while still inside the sterile pouch. Once the vial was sealed, it was removed from the pouch, and approximately 2mL sterile water was added to the vial using an 18-gauge sterile hypodermic needle. Hydration of the powder was then performed by subjecting the vial to ultrasonic agitation (QSonica) for 10-12hrs. Once hydrated, the hydrogel was transferred to laboratory refrigeration (T = 43C) for storage until use.

In vivo Model. Pigmented New Zealand rabbits (~2kg) 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). All animal research procedures were performed in accordance with IACUC, state, local and federal guidance for ethical treatment of animals.

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

Hydrogel Injection Tool. Hydrogel deployment was performed using a modified, sterile 1cc syringe. Approximately 0.3cc to 0.4cc 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 20mL syringe. The volume created between the 20mL syringe and the 1cc syringe was subsequently filled with a mixture of ammonium nitrate and water to induce an endothermic chemical reaction to cool the hydrogel during deployment, **Figure 3.3.1**. The endothermic reactants were given two minutes to react and bring the hydrogel to the desired temperature. Once ready, a modified, sterile intravenous, polymeric catheter tip was placed onto the end of the 1cc syringe and the hydrogel was deployed on the eye.

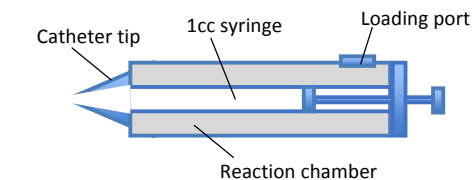


Fig 3.3.1. Schematic (top) and photograph (bottom) of engineered hydrogel injector tool with integrated, disposable temperature control system.

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

Post-Procedure Monitoring. Animals were checked regularly for signs of infection, discomfort or other adverse effects. Pain medication (ketofen 3mg/kg) was administered for 48hrs 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.

3.3.4 Results and Discussion.

A. Hydrogel can be deployed and seal 3mm penetrating injuries within ten minutes from procedure initiation. Once water is 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 $9^{\circ}C$, well below the LCST for this hydrogel formulation (LCST = 14-16C).

After two minutes, hydrogel deployment was initiated. In all instances when the injector tool's surface temperature was $T = 9^{\circ}C$, the hydrogel deployed smoothly and easily. Time-lapse photography images, **Figure 3.3.2**, show the process of hydrogel release into the incision. It was noted that the hydrogel is translucent on release into the eye. After only 30 seconds deployment the hydrogel begins 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 is completely opaque and beads of water can be seen on the surface. After five minutes, using surgical scissors, the excess hydrogel "cap" is 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).**

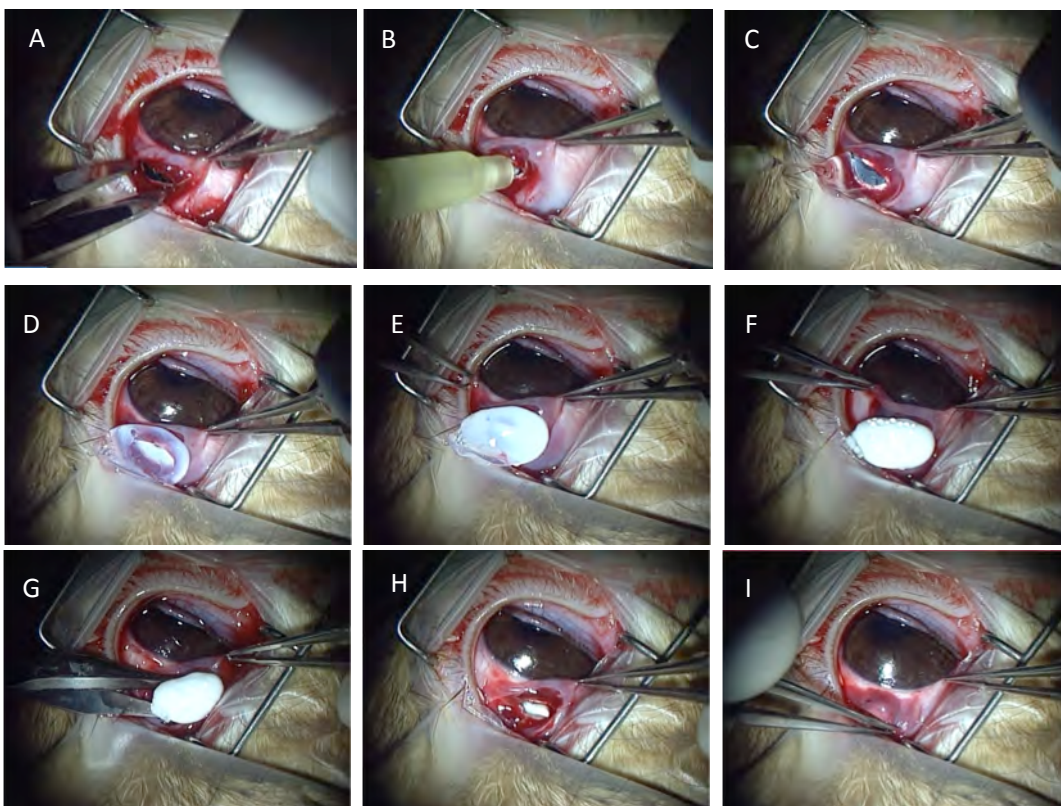


Fig 3.3.2. (top left to lower right): Time lapse photographs showing the stepwise procedure of hydrogel placement to seal a 3mm non-stellating scleral penetrating injury.

B. All treatment eyes (i.e. those that received the hydrogel) underwent an approximate 12hr - 24hr refractory period of low IOP ($IOP_{OD} = 2mm Hg$). Penetration of the scleral surface with the micro vitreoretinal blade causes 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 24hrs following the procedure, during which the ciliary epithelium of the eye produce aqueous humor to reestablish normal IOP. This is consistent with published rates of aqueous humor production from the eye¹.

C. Effective deployment of unsupported hydrogel requires 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, Figure 3.3.3. During each placement procedure the surgeon noted whether he felt like the hydrogel was properly deployed both into the posterior chamber, and through the perforation. In one instance (R-166) the surgeon reported his concern that perhaps not enough hydrogel was released into the posterior chamber. In one instance (R-171), the surgeon self-reported concerns that he excised too much material from the exterior cap of the hydrogel and that the hydrogel may fall into the posterior chamber.

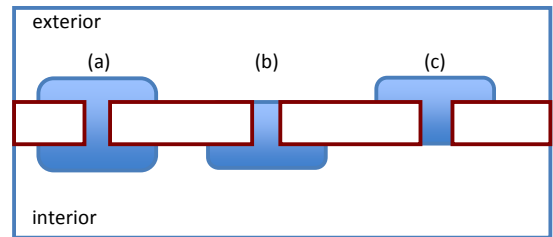


Fig 3.3.3. Schematic illustrating (a) ideal hydrogel placement through the scleral tissue, (b) not enough material placed on the exterior surface, allowing the patch to “fall into” the eye, and c) not enough material placed interior to the sclera allowing the patch to fall off.

D. Successful hydrogel placement into the scleral incision results in a post-refractory period average $IOP_{OD}/IOP_{OS} = 0.74 (\pm 0.09)$. To more accurately, assess pre-clinical performance of the hydrogel, the two predicted failures were separated from the treatment group and averaged with the no-treatment cohort, to account for deployment misplacement vs. actual adhesion performance. **Figure 3.3.4** plots average IOP_{OD}/IOP_{OS} of successful hydrogel placement (n=5) vs. control (n=5) **plus** failed hydrogel placement subjects (n=2). After only 7hrs of placement, the average of the treatment group relative $IOP_{OD}/IOP_{OS} = 0.61$ vs the contralateral and the average over the measured time period was 0.74 ± 0.09 . This was an average difference of over 50% vs eyes receiving no treatment.

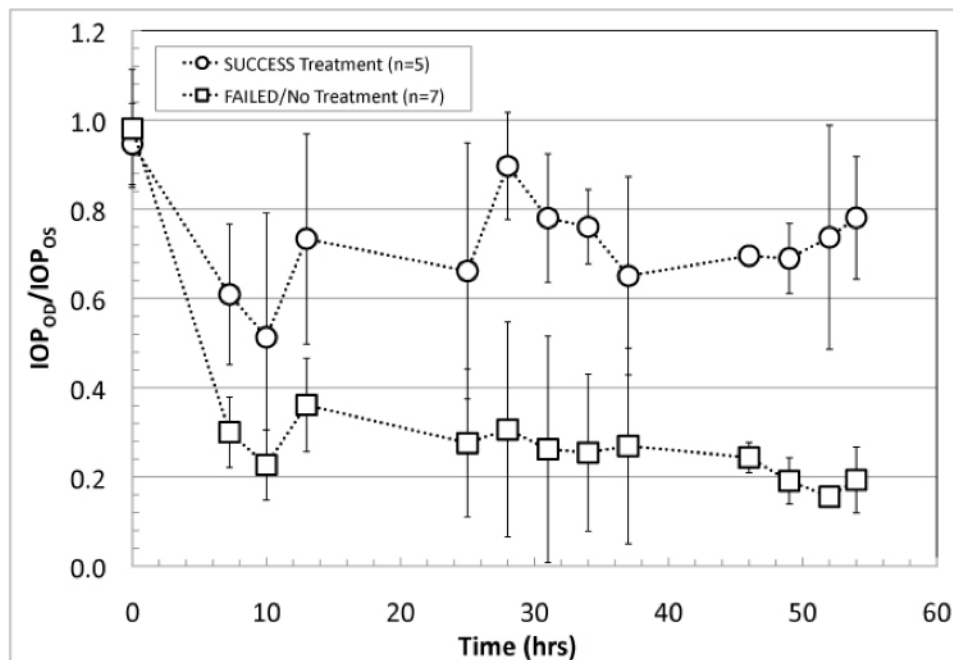


Fig 3.3.4. Comparison of IOP of eyes treated with hydrogel successfully, vs. those eyes receiving a similar penetrating injury and receiving either no treatment or hydrogel not properly placed. IOP of eyes receiving incision (OD) was normalized against contralateral eyes, which were left untouched (OS).

E. The hydrogel transitions from opaque to transparent after 48hrs implantation. After approximately 48hrs implantation, the hydrogel placement underwent a transformation from the opaque white color observed at t=0, to completely transparent and smooth after several hours, **Figure 3.3.5**. The exact timing of the transition has not been explored because evaluation requires additional sedation of the animals, potentially exceeding the allowed analgesia dose per 24hr period. The clarity of the hydrogel is such that visual inspection into the posterior chamber might be achieved.

At this stage it is unclear what the exact mechanism is that causes the transparency to develop. It is speculated that as individual water particles precipitate out of the hydrogel, they create small water particles with defined

boundaries that scatter light. After 48hrs these water particles are squeezed out of the hydrogel, leaving only the polymer in something similar to an annealed state with no internal grain boundaries that might scatter light.

F. Visual inspection of the hydrogel placement site shows little to no signs of irritation, Figure 3.3.6. Scleral tissue surround the implant sites show no signs of redness, inflammation or bleeding after 48hrs, suggesting that the hydrogel induces no adverse tissue response. Histological data is being collected to provide more critical analysis.

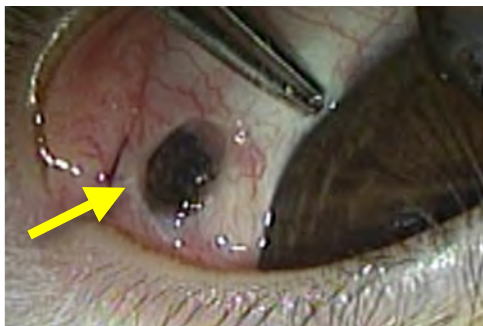
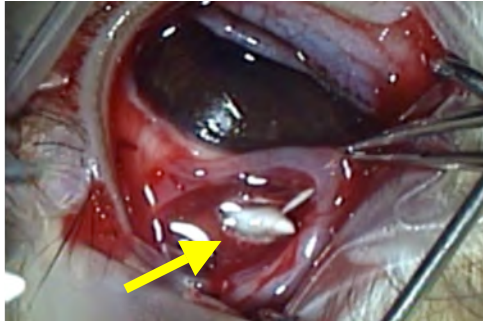


Fig 3.3.5. The hydrogel (yellow arrows) is opaque and white (top) at time of placement; and (bottom) hydrogel after 48hr placement appears smooth and transparent.



Fig 3.3.6 Optical micrographs of scleral surface showing hydrogel in situ after 48hrs.

3.4 In Vivo Peritomy Study.

This portion of the study has been delayed, and a no-cost extension was requested in August 2014 to allow for additional time to complete this milestone. Currently, patch hydrogel samples are being fabricated to initiate this effort. Rabbits have been ordered and scheduling has begun.

3.5 Histology.

3.5.1 Background.

Biocompatibility studies of PNIPAM hydrogel suggest that the polymer is biocompatible, or bioinert when implanted in the body²⁻⁴. The chemistry of the hydrogel used in these studies has been modified to create a block co-polymer composition of NIPAM with butylacrylate, therefore assessments should be performed to validate biocompatibility. The goal of this portion of the project is to assess local histological response of tissue to placement of hydrogel transcleral for 1-day, 7-days, and 30-days implantation to provide a preliminary assessment of biocompatibility.

3.5.2 Objectives.

- Deploy hydrogel into the right eye (OD) of rabbits to seal surgically created 3mm scleral penetrating injuries.
- Treated eyes will be monitored qualitatively for any signs of infection and irritation during implant period.
- At study endpoints, eyes will be enucleated and fixed for histological analysis.
- Staining will be performed to evaluate tissue reaction at the implantation site. Tissue response will be compared to eyes which have receive a similar 3mm incision but no intervention (wound was allowed to heal naturally).

3.5.3 Methods.

Surgical Procedure. Described previously in 3.3.3.

Implantation Duration. Endpoints at 1-day, 7-day, and 30-day will be evaluated.

Histology. After euthanization, the right eyes (OD) of animals were enucleated in one piece. Suture knots were placed around the implantation site to assist with proper tissue mounting and orientation for histology. Two small incisions were made into the sclera at opposite sides of the globe and away from the area of interest, to allow fixation solution to quickly fill the posterior chamber. Eyes were immersed in Davidson's solution for fixation and the appropriate tissue fixation protocol was followed.

Cross-sectional slices of tissue were prepared through the implantation location to view the hydrogel tract through the scleral tissue transversely. Hematoxylin and eosin staining was performed on selected samples for gross analysis of tissue response to the hydrogel. Other staining methods will be employed to more fully characterize histological response in the region of hydrogel placement.

3.5.4 Results and Discussion.

A. Histological comparison of hydrogel treated scleral penetrating injuries vs. naturally healed scleral penetrating injuries show no significant difference in immuno-response of adjacent tissue. Figure 3.5.1 compares 7-day histological tissue specimens from an eye treated with the hydrogel (left) vs. an eye with a similar penetrating injury but that received no intervention (right). Both tissue samples show a similarly sized tract through the sclera. The hydrogel was removed prior to tissue fixation, so it is not shown in the sample.

For the eye receiving the hydrogel, a complete encapsulation layer covers the fistula surface with no excessive signs of inflammation or irritation. In comparison, the cross-sectional image from the control eye shows that the fistula has been occluded by in-growth of tissue through the penetration. Boundaries of the scleral tissue around the fistula are not as clearly defined.

These are preliminary data based on a first series of histological slides. Additional data is being collected including immuno-staining to more fully characterize histological response to the foreign hydrogel material.

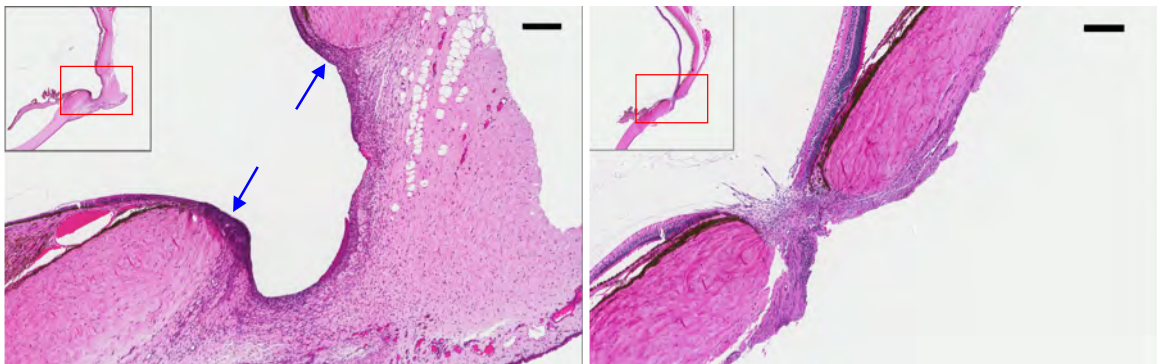


Fig3.5.1. (left) Histological cross-section of scleral fistula (edges marked by blue arrows) created by hydrogel placement after 7-days implantation, with low magnification (inset) provided for orientation. (right) 7-Day histological cross-section of scleral fistula that was allowed to heal without intervention (control), showing tissue in-growth closing fistula. Scale bars (top right of each image) are 200µm.

4. Key Research Accomplishments

In year two of this program the following key research accomplishments were achieved:

4.1 New Strategies Defined.

- A. ***An effective unsupported hydrogel formulation has been developed.*** Unsupported hydrogel formulations based on copolymers of PNIPAM with butylacrylate with adhesion characteristics comparable to cyanoacrylate were identified and adhesion characterized via IOP testing. Work continues on developing a supported substrate patch.
- B. ***Revised IOP Test System Designed and Validated.*** While the cadaveric IOP test returned irregular measurements, a more controlled IOP test system employing dissected pieces of cadaveric scleral tissue was designed and validated. Test results look significantly more repeatable compared to the cadaveric eye tests.
- C. ***A prototype injector tool for hydrogel delivery has been designed, fabricated and validated.*** A disposable tool designed from syringes and using ammonium nitrate powder and water to generate an endothermic reaction, was designed and repeatedly used to deploy hydrogel in vivo successfully.

4.2 In Vitro Characterization Accomplishments

- A. ***Unsupported PNIPAM:butylacrylate co-polymer hydrogel is able to maintain IOP values as high as 70mm Hg.***

4.3 In Vivo Testing Validation

- A. ***Unsupported hydrogel has been successfully implemented in vivo in a rabbit model.*** IOP increases to 60% of normal within 12 hours of placement. In comparison, average IOP of traumatized eyes receiving no hydrogel maintain only 25% of normal IOP for 7 days.
- B. ***Preliminary histology suggests no significant tissue response to implantation after 48hrs.*** Histology slides from eyes implanted 48hrs show no significant irritation or inflammation indicative of material rejection.

5. Conclusions.

This study aims to validate a novel biomaterial for temporary treatment of traumatic injuries to the eye. The intended use is within the first few minutes to hours of sustaining an injury, with the objective of reestablishing intraocular pressure as soon as possible. ***With respect to medical significance, this work is significant because no comparable technology exists today for rapid intervention of scleral trauma that offers equivalent ease of use and reversibility of attachment.***

To date, we have demonstrated the following:

- A. The hydrogel adhesive performance is substantially equivalent to cyanoacrylate, a commonly used, irreversible medical adhesive.
- B. We have demonstrated the ability to reestablish 75% of normal IOP within 12 hours of hydrogel placement, without using any other intervention. This is greater than 50% higher IOP vs. traumatized eyes left untreated.
- C. Preliminary qualitative observations of hydrogel-treated eyes show no significant adverse reaction to hydrogel placement, suggesting bioinertness within the time of use.

With respect to military significance, the incidence of ocular trauma has significantly increased in the last several years as a result of changes in warfare strategies, and clinical intervention strategies involve three steps: 1) immediate stabilization of the casualty by nearby medics, 2) transportation and emergency intervention at the nearest combat service hospital, and lastly 3) complete surgical treatment leverage full facilities at a base hospital. Steps 2 and 3 result in sustained hypotony (low IOP) for greater than 24hrs, increasing the probability of retinal ischemia and potential vision loss. ***Preliminary data suggests placement of the hydrogel near the time of injury reestablishes IOP to near normal levels within 12 hours, thus potentially reducing the risk of retinal ischemia in the injured warfighter.***

6. Publications, Abstracts and Presentations

Y. Zhang, P. Falabella, N. Bayat, S. Rauen, J.D. Weiland, J.J. Whalen, M.E. Thompson, M.S. Humayun. "Thermoresponsive Reversible Adhesive for Temporary Intervention in Ocular Trauma." ARVO Annual Meeting. Orlando, FL. May 4-8, 2014.

7. Inventions Patents and Licenses

US Patent Application 61/934,061: System for Sutureless Closure of Scleral Peforations and other Ocular Tissue Discontinuities. J.J. Whalen III, N. Bayat, Y. Zhang, P. Falabella, M.E. Thompson, M.S. Humayun. Filing Date: 31-JAN-2014.

8. Reportable Outcomes

Nothing to report.

9. Other Achievements

Dr. Zhang's ARVO May 2014 abstract was selected to be honored as an Emerging Trends and Hot Topics Abstract: http://www.arvo.org/uploadedFiles/ARVOORG/Annual_Meeting/2014/Hot%20Topics_2014.pdf

10. References

1. M. Goel, R.G> Picciani, R.K. Lee, S.K. Bhattacharya. "Aqueous Humor Dynamics: A Review." Open Ophthalmol J. 4 (2010) 52-59.
2. M.A. Cooperstien, H.E. Canavan. "Assessment of Cytotoxicity of (N-Isopropylacrylamide) and N-isopropylacrylamide surfaces." Biointerphases (2013) 8:19.
3. Z. Cui, B.H. Lee, C. Pauken, B.L. Vernon. "Degradation, cytotoxicity, and biocompatibility of NIPAAm-based Thermosensitive, injectable, and bioresorbable polymer hydrogels. J. Biomed Mat. Res. A. Aug 2011 v. 98A. 159-166.
4. L. Zou, A. Nair, H. Weng, Y-T Tsai, Z. Hu, L. Tang. "Intraocular Pressure Changes: An Important Determinant of the Biocompatibility of Intravitreal Implants." PLoS One. Dec. 2011. V. 6 issue 12. E28720. 1-9.

11. Appendices

- A. USPTO Provisional Application Filing
- B. Y. Zhang et al. ARVO 2014 Abstract.

Appendix A. USPTO Filing Receipt



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Table with 6 columns: APPLICATION NUMBER, FILING or 371(c) DATE, GRP ART UNIT, FIL FEE REC'D, ATTY. DOCKET NO, TOT CLAIMS, IND CLAIMS. Row 1: 61/934,061, 01/31/2014, 130, 028080-0975

CONFIRMATION NO. 1161

FILING RECEIPT

33401
McDermott Will & Emery LLP
The McDermott Building
500 North Capitol Street, N.W.
Washington, DC 20001



Date Mailed: 03/19/2014

Receipt is acknowledged of this provisional patent application. It will not be examined for patentability and will become abandoned not later than twelve months after its filing date. Any correspondence concerning the application must include the following identification information: the U.S. APPLICATION NUMBER, FILING DATE, NAME OF APPLICANT, and TITLE OF INVENTION. Fees transmitted by check or draft are subject to collection. Please verify the accuracy of the data presented on this receipt. If an error is noted on this Filing Receipt, please submit a written request for a Filing Receipt Correction. Please provide a copy of this Filing Receipt with the changes noted thereon. If you received a "Notice to File Missing Parts" for this application, please submit any corrections to this Filing Receipt with your reply to the Notice. When the USPTO processes the reply to the Notice, the USPTO will generate another Filing Receipt incorporating the requested corrections

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Permission to Access - A proper Authorization to Permit Access to Application by Participating Offices (PTO/SB/39 or its equivalent) has been received by the USPTO.

If Required, Foreign Filing License Granted: 03/18/2014

The country code and number of your priority application, to be used for filing abroad under the Paris Convention, is US 61/934,061

Projected Publication Date: None, application is not eligible for pre-grant publication

Non-Publication Request: No

Early Publication Request: No

**** SMALL ENTITY ****

Title

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

Statement under 37 CFR 1.55 or 1.78 for AIA (First Inventor to File) Transition Applications: No

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Appendix B. ARVO 2014 Abstract

CONTROL ID: 1919837

TITLE: Thermoresponsive Reversible Adhesive for Temporary Intervention in Ocular Trauma

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Commercial Relationships Disclosure (Abstract): Yi Zhang: Commercial Relationship: Code N (No Commercial Relationship) | Paulo Falabella: Commercial Relationship: Code N (No Commercial Relationship) | Niki Bayat: Commercial Relationship: Code N (No Commercial Relationship) | Stacey Rauen: Commercial Relationship: Code N (No Commercial Relationship) | James Weiland: Commercial Relationship: Code N (No Commercial Relationship) | John Whalen: Commercial Relationship: Code N (No Commercial Relationship) | Mark Thompson: Commercial Relationship: Code N (No Commercial Relationship) | Mark Humayun: Commercial Relationship: Code N (No Commercial Relationship)

Study Group:

ABSTRACT BODY:

Purpose: Poly(N-isopropylacrylamide), pNIPAM, is a temperature-responsive polymer which exhibits a reversible macromolecular transition that demonstrates adhesive properties at body (eye) temperature and non-adhesive properties at decreased temperature. We report on the feasibility of developing a novel tissue adhesive to seal scleral wounds using pNIPAM-based gels.

Methods: Free-standing pNIPAM-based gels 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. An in vitro cadaveric porcine eye model was designed to assess the ability of the gels to seal penetrating incisions through the sclera, mimicking clinical cases. Adhesion in each test was compared against medical-grade cyanoacrylate glue and sutures, respectively. Real-time IOP was tracked in the whole porcine eye using 19-gauge catheter pressure transducer inserted through the pars plana. Primary objectives: 1) Determine whether pNIPAM-based gel can reach adhesion performance of cyanoacrylate in uniaxial tension testing. 2) Determine if pNIPAM-based gel can prevent hypotony in a cadaveric porcine eye and maintain IOP comparable to suture. Secondary objective: Determine if pNIPAM-based gel can be removed using a temperature lowering protocol.

Results: Synthesized pNIPAM-based 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 70-77mm 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.

Conclusions: pNIPAM-based gel adhesives may offer a fast and reversible approach to temporarily and satisfactorily seal scleral penetrations. Such adhesives represent a promising new reversible technique for temporary intervention in ocular trauma and other applications.

(No Image Selected)

PRESENTATION TYPE: Poster Only

CURRENT * REVIEWING CODE: 2583 ocular therapeutics and chemical biology - BI

CURRENT * SECTION: Biochemistry/Molecular Biology

KEYWORDS: 765 wound healing, 708 sclera, 742 trauma.

Clinical Trial Registration: No

Other Registry Site:

Registration Number:

Date Trial was Registered (MM/DD/YYYY) :

Date Trial Began (MM/DD/YYYY):

Grant Support: Yes

Support Detail: TATRC Grant W81XWH-10-2-0076

TRAVEL GRANTS and AWARDS APPLICATIONS

AWARDS: