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

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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 cyanoacrylate glue, a commonly used medical adhesive. Once successful adhesion performance is completed in vitro, adhesion in vivo and biocompatibility will be assessed using a rabbit animal model.					
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Executive Summary.

This project titled, "VRPI Thermoresponsive Reversibly Attachable Patch for Temporary Intervention in Ocular Trauma", is a two-year program to develop a novel, sutureless wound closure technology to treat penetrating and perforating injuries to the eye. The enabling technology is a polymer called poly(N-Isopropyl acrylamide), or pNIPAM, a thermo-responsive polymer that adheres to ocular tissues at body temperature, and detaches from the tissue when exposed to cooler temperatures.

Our year 1 objectives were to complete in vitro characterization of pNIPAM scleral patches. This included: assessment of effects of medical device sterilization protocols and extreme temperature exposure on adhesion performance, and characterization of strength of adhesion to scleral tissue, using in vitro tissue models. Successful completion of these tests would enable in vivo studies in year 2.

The following key findings have been accomplished or demonstrated, to date:

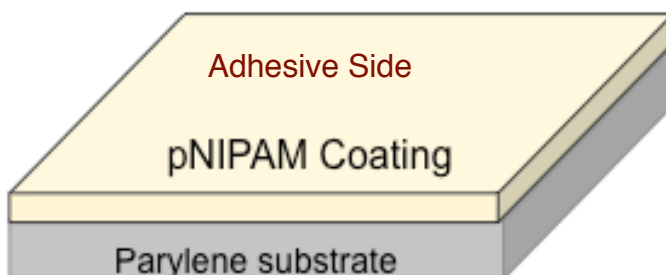
1. pNIPAM-on-parylene scleral patches can be fabricated using either an Atom Transfer Radical Polymerization (ATRP) process or Chemical Vapor Deposition (CVD).
2. Both a uniaxial adhesion strength test and an in vitro IOP model test have been created and validated for measuring scleral patch adhesion to tissue.
3. The adhesion strength of pNIPAM to scleral tissue is directly proportional to the aqueous concentration of the pNIPAM formulation.
4. Using the uniaxial pull test, the 43.2% pNIPAM aqueous solution exhibits 80% of the adhesion strength exhibited by cyanoacrylate, when tested in the uniaxial test protocol.
5. In the IOP porcine eye test using a 3mm sclerotomy incision, the 43.2% pNIPAM aqueous solution reduced sclerotomy leak rate from 19cc/min to 12cc/min at 16.5mm Hg pressure.
6. In the uniaxial pull test, the 10% aqueous solution of (85%:15%) [pNIPAM:n-tert butylacrylamide] exhibits an adhesion force approximately 80% that of cyanoacrylate.
7. In the IOP porcine eye test using a 3mm sclerotomy incision, the 10% aqueous solution of (85%:15%) [pNIPAM:n-tert butylacrylamide] shows a leak rate of 7.5cc/min at 20mm Hg and 5cc/min at 10mm Hg pressure.
8. Additional work is being conducted to prepare different pNIPAM-pNTBAM co-polymer formulations to completely arrest sclerotomy leakage in the IOP test.

Conclusions: At this stage, the stop-gate for moving to in vivo studies is complete elimination of the saline leak from the patched sclerotomies in the IOP test. We have demonstrated adhesions strengths equal to approximately 80% the adhesion strength of cyanoacrylate to sclera (uniaxial pull test), however the leak rate tests in the IOP model test suggest that adhesion must be increased before moving to the in vivo models.

Introduction.

This project titled, "VRPI Thermoresponsive Reversibly Attachable Patch for Temporary Intervention in Ocular Trauma", is a two-year program to develop a novel, sutureless wound closure technology to treat penetrating and perforating injuries to the eye. The enabling technology is a polymer called poly(N-Isopropyl acrylamide), or pNIPAM, a thermo-responsive polymer that adheres to ocular tissues at body temperature, and detaches from the tissue when exposed to cooler temperatures.

Patches are fabricated by depositing pNIPAM onto a bio-inert polymer substrate via one of two processes: either a wet chemical synthesis called atom transfer radical polymerization or via a chemical vapor deposition (CVD) process. In both cases the adhesive polymer is mated to a parylene substrate creating a "band-aid like" adhesive patch, Figure 1.



The first year of this program focused on patch fabrication using different pNIPAM processing conditions to identify a preferred formulation that: 1) provides sufficient tissue adhesion characteristics to maintain normal intraocular pressure (IOP), and 2) survives standard medical device sterilization protocols and material transport temperature exposure protocols.

Figure 1. Schematic illustrating the structure of the PNIPAM patches with pNIPAM coating (100-800nm) deposited onto a 10-micron parylene substrate.

Patches that meet all prescribed engineering and performance criteria will be identified and used in year 2 for in vivo (rabbit) safety and efficacy studies.

Five (5) tasks were approved for the year 1 statement of work for this project:

Table 1. Statement of Work Tasks	
Task	Quarter
Fabricated test patches	Q1
Sterilize test patches	Q1
Temperature exposure of patches	Q1
Adhesion performance characterization	Q3
Time to attach/detach test	Q4

The following is a synopsis of the key findings and accomplishments for year 1, as they relate to these five tasks.

Body.

Task 1 (Q1): Fabricate Test Patches.

Research Accomplishment 1: *pNIPAM test patches have been successfully fabricated using both chemical synthesis approach, and vapor deposition approach.*

Parylene was chemical vapor deposited (thickness = 10 micron) on silicon wafers to create the patch substrates, Figure 1. pNIPAM was subsequently deposited over the parylene using either wet chemical synthesis approach or a chemical vapor deposition (CVD) process, depending on the final pNIPAM thicknesses.

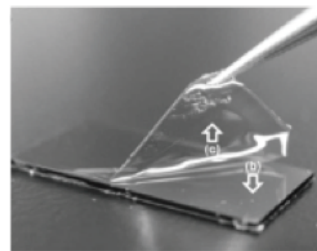


Figure 1. CVD deposited parylene-C on silicon substrate.

Atom Transfer Radical Polymerization (ATRP) was the first approach used to fabricate patches. A two-step process, Figure 2, is used to grow via the aqueous ATRP approach. No crosslinkers are used in this approach because each chain is initiated from a surface-bound activator site.

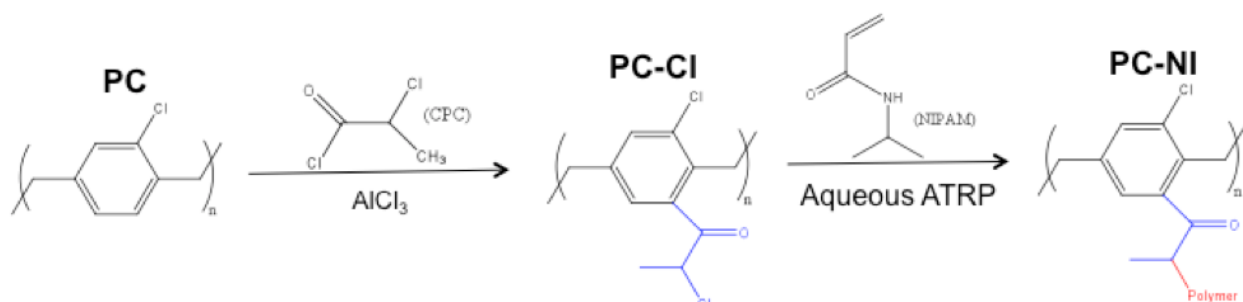


Figure 2. ATRP synthesis route for pNIPAM.

Chemical Vapor Deposition (CVD), illustrated in Figure 3, was also used to fabricate samples with thicker pNIPAM. CVD flows acryloyl chloride monomer inside of a vacuum chamber over parylene substrates that have been functionalized with vinyl groups on their surface. The rate of growth is controlled by the rate of flow of monomer and reactants. The CVD approach enables thicker deposits (e.g. 200nm to 1,000nm) of pNIPAM.

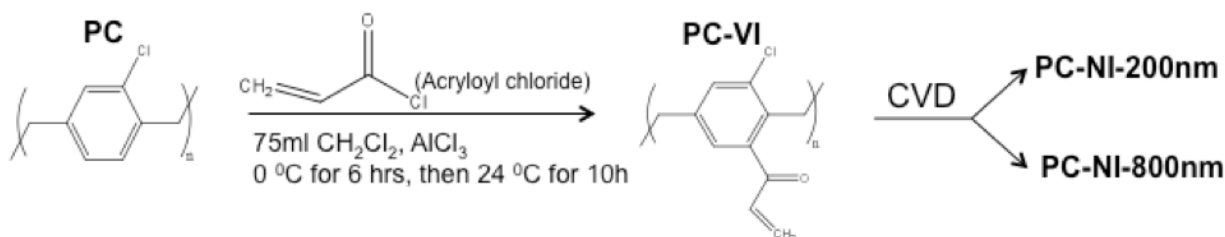


Figure 3. Route for CVD synthesis of pNIPAM

Following deposition, both patch types must be rinsed in de-ionized water to remove unpolymerized monomer. Some data suggests that unpolymerized NIPAM is cytotoxic with increasing quantity¹. Figure 4 is a photograph taken of a finished ATRP pNIPAM-parylene patch. CVD-based patches were prepared using conditions prescribed for growing pNIPAM thicknesses of 200nm, 400nm and 800nm.

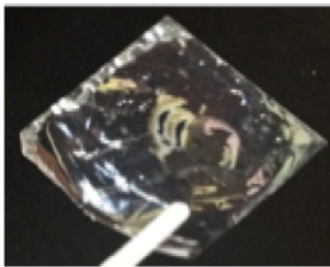


Figure 4. ATRP synthesized pNIPAM-parylene patch.

Film Thickness Measurements. CVD deposited PNIPAM requires crosslinking additives to maintain thickness and adhesion to substrate. Ellipsometry measurements were performed on pNIPAM-on-substrate samples post-deposition and again post-rinsing to measure film thickness. ATRP synthesized samples showed no measurable difference in thickness (data not shown). In contrast a significant decrease in pNIPAM film thickness was observed after the rinsing process, Table 2.

Table 2. ATRP Deposited pNIPAM Thickness

Targeted Thickness	Ellipsometry Measurements	
	Before Rinsing	After Rinsing
100 nm	109.0 $\pm$ 4.4	9.7 $\pm$ 2.1
400 nm	440.3 $\pm$ 38.4	7.7 $\pm$ 1.5
800 nm	897.0 $\pm$ 21.7	7.0 $\pm$ 1.0

These data suggest that pNIPAM deposited by CVD has a lower strength of attachment to the base pNIPAM-parylene, and therefore some approach must be used to improve interfacial strength between CVD pNIPAM and the substrate. Cross-linking groups were added to the process to network pNIPAM chains in the film together and improve anchoring. Studies of this are ongoing.

Research Accomplishment 2: Block co-polymer patches of pNIPAM with n-tert Butylacrylate polymer have been fabricated to further enhance adhesion.

NIPAM, N-tert-butylacrylate and 2,2'-azo-bis-isobutyronitrile were dissolved in dry tetrahydrofuran and benzene, and reacted through the same ATRP process to create (85% NIPAM 15% N-tert-butylacrylate). Addition of the hydrophobic N-tert-butylacrylate (LCST = 10°C) lowers the LCST of the resulting block co-polymer². Our hypothesis now is that lowering the LCST of the co-polymer will increase the hydrophobicity and subsequently increase the attraction between the polymer and tissue.

Task 2 (Q1): Sterilize Patches.

Research Accomplishment 3: We have successfully demonstrated that a standard medical device industry ETO sterilization protocol causes no adverse effects to chemistry or performance. Both Fourier Transform Infrared Spectroscopy (FTIR) and contact angle measurements performed on samples detect no change following ETO sterilization.

Patches of each type (ATRP and CVD) were either exposed to a standard ETO sterilization protocol or standard autoclave (pressurized steam) sterilization protocol. We used a standard

ETO sterilization protocol that exposed samples to vaporized ethylene oxide gas at 55°C at a concentration between 400-800mg/L for 3 hours. The *autoclave sterilization* protocol exposed samples to steam heated to between 130°C for 15 minutes followed by cooling.

Samples were packaged in separate, polyethylene mesh bags by pNIPAM thickness. Sterilization completion was confirmed by visual indicators on each bag. Post-sterilization, patches were re-characterized by FTIR and contact angle to look for changes.

Fourier Transform Infrared Spectroscopy (FTIR)

was used to detect chemical structure changes caused by sterilization. Both ATRP and CVD spectra were first compared against a control sample (purchased) to confirm structure (data not shown). Figure 5 is a representative plot comparing results from an ATRP synthesized pNIPAM patch sample, pre- and post- ETO sterilization. The amide groups of our pNIPAM coated substrates give characteristic stretches, due to C=O stretching (1640 cm^{-1} , amide-I mode) and a combination of C-N stretching and N-H bending (1550 cm^{-1} , amide-II mode). The amide stretches are the best way to probe for damage in the film that may be caused by our sterilization procedures and will be used below to show that the pNIPAM films are completely stable to both ethylene-oxide and autoclave sterilization (1999 Ref). Here we measured amide-I mode before and after sterilization and thermal exposure. FTIR spectra taken pre-sterilization and post-sterilization show no statistical difference before and after treatment.

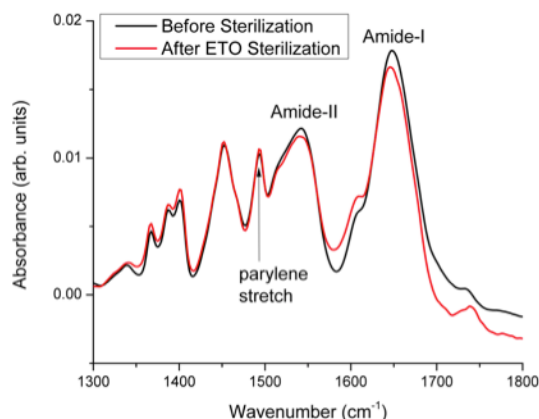


Figure 5. FTIR spectra for ATRP-synthesized pNIPAM patches, pre-sterilization (black) and post-sterilization (red).

Currently, work is ongoing in changing the polymer chemistry. FTIR studies will be repeated once the pNIPAM synthesis approach has been locked.

Contact Angle (CA) Measurements were also used to evaluate the effect of sterilization. Contact angle measurements characterize the hydrophobicity/philiicty of a polymer. As hydrophobicity increases, so does the contact angle. Typically, a sample of the test material is placed onto a substrate and a single angle measure is recorded. Here, contact angle was measured over a temperature range around the Lower Critical Solution Temperature (LCST). The LCST is the temperature below which pNIPAM becomes hydrophilic and above which it becomes hydrophobic.

Measurements taken pre-sterilization and post-sterilization, Figure 6 (a and b) on the next page, showed no measurable difference in contact angle measure over the LCST range. Over the temperature range $T = (10^{\circ}\text{C} \text{ to } 50^{\circ}\text{C})$ the CVD samples demonstrated a larger hydrophobic/philiic switch ($\Delta\text{CA} = 50^{\circ}$) vs. the ATRP ($\Delta\text{CA} = 30^{\circ}$) samples.

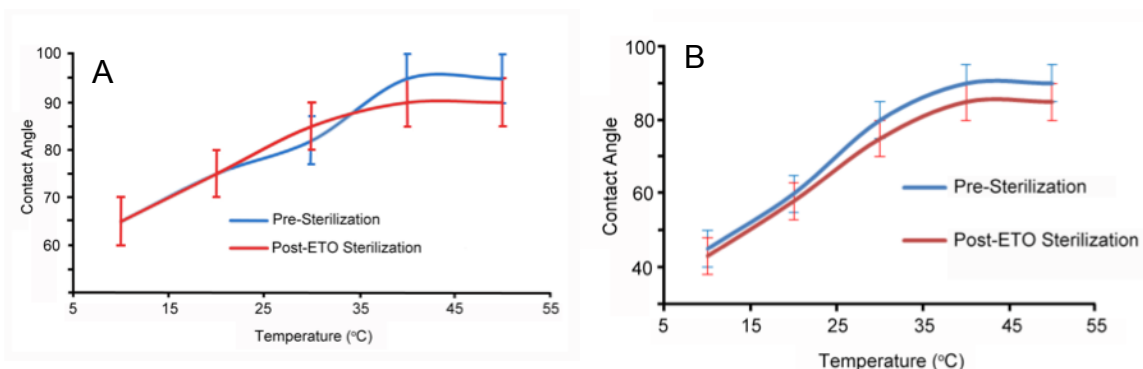


Figure 6. Contact angle vs. temp measurements pre-sterilization (blue) and post-sterilization (red), for (A) ATRP-synthesized PNIPAM on parylene patches, and (B) CVD-synthesized PNIPAM on parylene patches.

Task 3 (Q1): Temperature Exposure of Patches

Research Accomplishment 4: *Contact angle and FTIR data suggest that exposure to both high ($T = 120F$) and low ($T = -46F$) for 72hr period do not effect chemical structure or performance.* More studies will be performed once the copolymer chemistry is finalized.

We have designed a temperature exposure protocol using MIL-STD-810G guidance for high temperature and low temperature exposure. These protocols (described below) were designed based on input from TATRC advisors and literature, and are used to provide a preliminary assessment of high temperature and low temperature exposure during transportation.

High Temperature Protocol Key Characteristics*

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

Low Temperature Protocol Key Characteristics*

- Duration: 3 Day
- Thermal protocol: Constant Temperature
- Temperature Exposure: -46F
- Test samples are placed inside simulated packaging
- Thermal Chamber

Further temperature exposure testing will be continued once the block copolymer chemistry and fabrication route is finalized. At this stage we are reasonably confident that any polymer formulation within the compositions we are working with should survive testing. These studies will be repeated once the pNIPAM polymer chemistry is finalized.

Task 4 (Q3): Adhesion Performance Characterization (Q3)

Research Accomplishment 5: *A linear pull testing protocol to measure tissue-patch adhesion strength was created, tested and validated.* Please see Appendix for protocol development background as well as protocol description. The system is based on a Bose uniaxial tension tester.

Adhesion to scleral tissue was tested by sandwiching unsupported (no parylene) pNIPAM between two pieces of dissected scleral tissue that were fixated to the base and actuator arm of the pull tester, respectively, see Figure 7. Apposed tissue were brought down and pressed together using 15g of pressure for 2 minutes and then the actuator began the pulling procedure until the two tissue samples pulled apart.

Figure 8 compares the maximum adhesion strength recorded for the best performing pNIPAM formulation prepared, against the maximum adhesion strength for cyanoacrylate glue using the same test setup.

These results show that the average maximum adhesion strength for cyanoacrylate glue fixed to scleral tissue was approximately 260mN. It also shows that the 43.2% pNIPAM solution yielded a maximum adhesion strength of approximately 210mN. This is less than 20% short of matching the performance of cyanoacrylate in the uniaxial pull test.

More pull tests will have to be performed to generate statistically significant data comparing the adhesion strengths. But at this stage we are reasonably confident that the normal adhesion strength of the patches will match cyanoacrylate performance.

Figure 9 plots the series of adhesion strength measurements taken from the different pNIPAM aqueous concentrations that were prepared over the past 10 months. A trend can clearly be seen, namely, increasing adhesion strength with increasing pNIPAM concentration. Our goal is to continue raising pNIPAM concentration until the saturation becomes too high to maintain in solution.

Figure 9. (right) Plot of maximum adhesion strengths of different pNIPAM formulations.

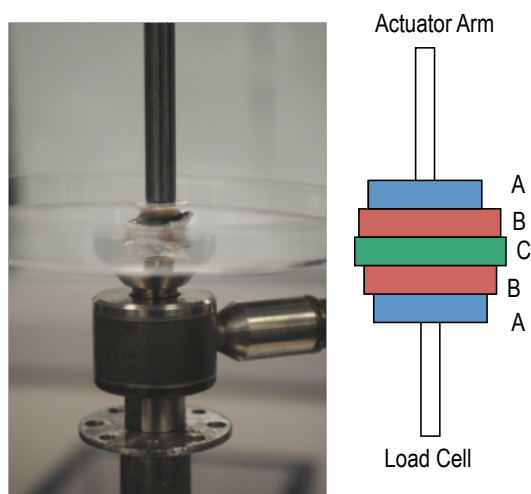


Figure 7. Image (left) and schematic (right) of uniaxial pull test system. Stack between actuator arm and load cell consisted of (A) arm base, (B) scleral tissue, (C) pNIPAM sample.

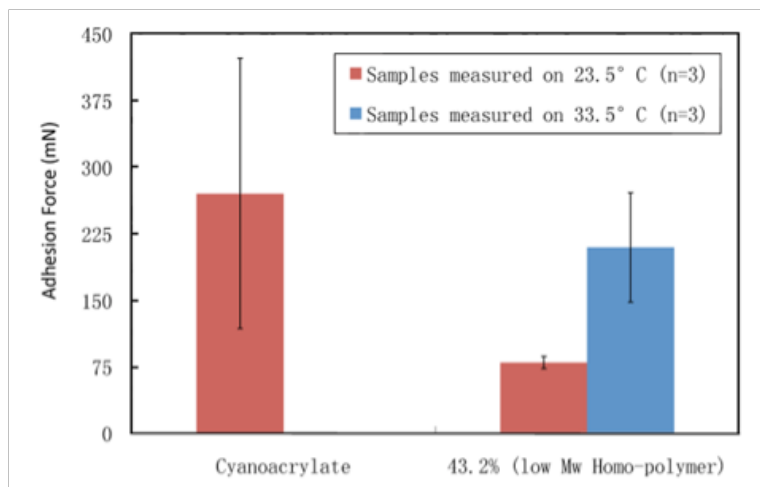
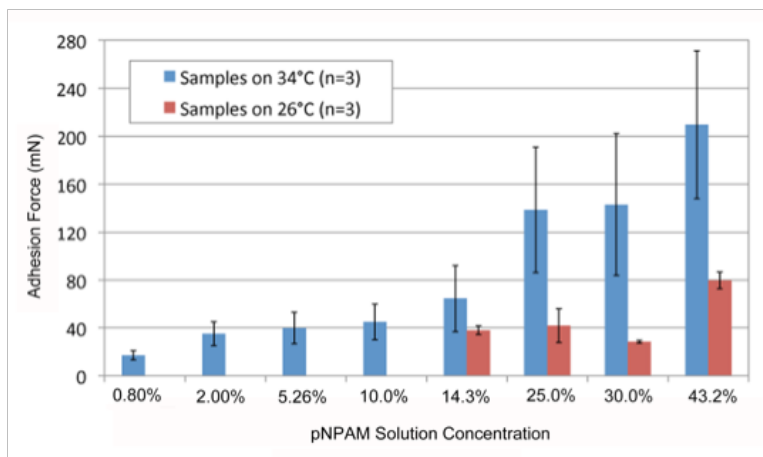


Figure 8. (above) Adhesion strength of cyanoacrylate to scleral tissue (left), compared against adhesion strength of 43% aqueous solution pNIPAM (right) at room temperature (red) and body temperature (blue).



Research Accomplishment 6: *pNIPAM and pNIPAM-n-tert butylacrylamide copolymer formulations were synthesized and we have identified a chemistry approaching the linear pull attachment strength of cyanoacrylate. More work is needed and ongoing.*

Figure 10 (right) shows preliminary adhesion strength measurement data on a new block co-polymer (85% pNIPAM and 15% N-tert butylacrylamide) in a 10% aqueous suspension. Here we see that at 33.5°C the average adhesion strength is approximately 200mN.

n-tert butylacrylamide (LCST = 10°C) is more hydrophobic at body temperature vs. pNIPAM (LCST = 32°C), therefore we anticipate an improved adhesion.²

For comparison, we have included in Figure 10 (left) the adhesion strength data for the 10% homo-polymer (i.e. pure pNIPAM) aqueous solution. If we assume the co-polymer will have similar behavior to the homo-polymer, we can extrapolate that a 20%-30% aqueous suspension of the block co-polymer should exceed the adhesion performance of cyanoacrylate.

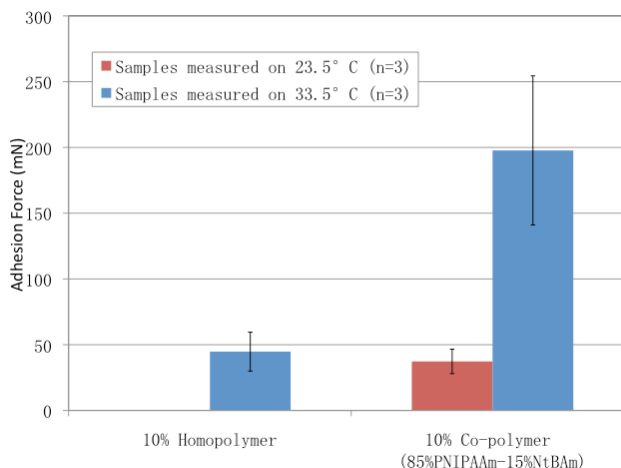


Figure 10. Comparison of adhesion strength of 10% pNIPAM homo-polymer and 10% (pNIPAM-n tert butylacrylamide (85:15%)).

Progress to prepare higher aqueous concentration block co-polymer, and to test them, is ongoing.

Research Accomplishment 7: *An in vitro model to test adhesion strength as a function of intraocular pressure (IOP) was designed, tested and validated.* Details regarding this protocol are located in the Appendix.

Research Accomplishment 8: *In vitro IOP test results suggest that greater adhesion forces are required to prevent leakage in the anatomical model (cadaveric porcine eye), than the linear pull test.* pNIPAM and pNIPAM- n-tert butylacrylamide copolymer formulations were tested for ability to prevent posterior chamber leakage and maintain IOP. Higher aqueous concentration block co-polymer formulations are being synthesized and tested now.

IOP tests were conducted in four stages to collect four measurements for each patch. Those four measurements, shown in Figure 11, were: I.) Saline infusion flow rate at baseline, II.) saline infusion flow rate after creation of a linear, fully penetrating incision of the sclera (l = 2-3mm), III.) saline infusion flow rate after suturing the incision closed, and IV.) saline infusion flow rate after removing the sutures and attempting closure with the pNIPAM patch.

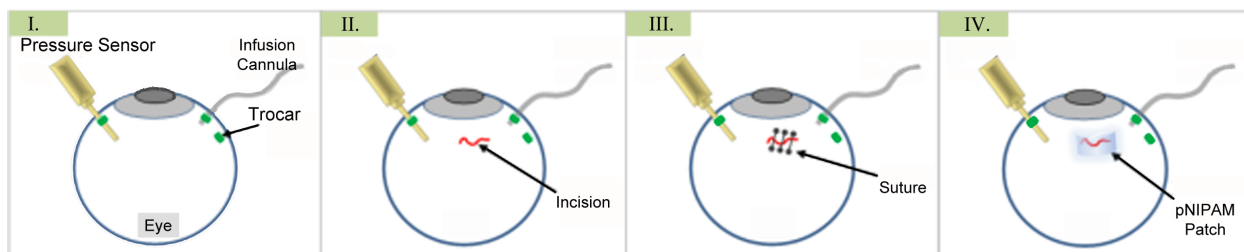


Figure 11. Schematic diagrams depicting the four stages of the in vitro IOP tests.

Table 3. Saline Infusion Rates in Porcine Eye Model of Scleral Penetration				
Adhesive Material Tested	Infusion Flow Rate (cc/min)			
	I.	II.	III.	IV.
Cyanoacrylate Glue	0	18	0	0
43.2% pNIPAM at 16.5 mm Hg	0	19	0	12
10% (85% pNIPAM: 15% n-tert ButylA) 10mm Hg	0	10	0	5
10% (85% pNIPAM: 15% n-tert ButylA) 20mm Hg	0	13	0	7.5

Table 3 lists the flow rates recorded for cyanoacrylate along with two sample patches (one measured at two different IOP magnitudes) with the best performance to date.

Stages I, II and III allowed us to validate that the experimental setups were working properly by showing that flow was non-existent at baseline, that flow was high upon creation of the penetrating incision, and that flow was completely arrested upon suture placement.

Column IV are the values of merit in Table 3. The preliminary results we have gathered on the homo-polymer at 16.5mm Hg and co-polymer at 10mm and 20mm Hg suggest that the adhesion is not completely sealing the eye under physiological pressure. In comparison, we have seen complete arrest of saline flow rate by cyanoacrylate.

We are continuing to explore different polymer chemistries to improve the adhesion strength of the patch to tissue, to further decrease the measured flow rates.

Task 5 (Q4): Time to Attach/Detach Test

Research Accomplishment 9: *We have developed a protocol to measure pNIPAM attachment/detachment from scleral tissue.* These data will be captured once we have finished the uni-axial and IOP adhesion testing on the block co-polymer chemistries.

Key Research Accomplishments.

1. Demonstrated ability to fabricate patches both via wet chemical synthesis and using CVD process.
2. Confirmed that sterilization has no effect on adhesion performance of pNIPAM patches (contact angle and FTIR data).
3. Confirmed that extreme temperature exposure has no effect on adhesion performance of pNIPAM patches (contact angle and FTIR data).
4. Scleral patch adhesive composition has been narrowed to a smaller composition profile, however more work is needed to determine the exact composition and synthesis route for this application.
5. Scleral patch uniaxial adhesive strength has almost reached the same performance as cyanoacrylate glue (pNIPAM strength = 80% cyanoacrylate)
6. Scleral patch adhesion performance in IOP test currently does not meet performance criteria (it is allowing unacceptable amount of saline leak from incisions during in vitro, cadaveric porcine eye test).
7. Different adhesive polymer chemistries are being tested in IOP study to eliminate leak rate.
8. Preparing for animal studies in year two.

Reportable Outcomes.

Publications/Presentations: Public disclosures of finding on this project have been withheld in the first half of year 1, to protect proprietary information on which we will be filing patents. We are currently preparing a disclosure which should be reported in upcoming quarters.

Two abstracts are being prepared for submission in the next 3-6 months (one in ophthalmology and one in chemistry/chemical engineering).

Degrees obtained that are supported by this award: N/A

Additional Funding applied for: N/A

Employment or research opportunities applied for or received based on experience/training from this award: N/A

We have not applied for additional funding using the results of this study. However, we have engaged in preliminary dialogue with a potential commercialization partner that is active in the ocular surgery instrumentation space. Commercialization efforts will be initiated, pursuant to successful completion of this program's tasks .

Conclusion.

1. Demonstrated ability to fabricate patches both via wet chemical synthesis and using CVD process.
2. Confirmed that sterilization has no effect on adhesion performance of pNIPAM patches (contact angle and FTIR data).
3. Confirmed that extreme temperature exposure has no effect on adhesion performance of pNIPAM patches (contact angle and FTIR data).
4. Scleral patch adhesive composition has been narrowed to a smaller composition profile, however more work is needed to determine the exact composition and synthesis route for this application.
5. Scleral patch uniaxial adhesive strength has almost reached the same performance as cyanoacrylate glue (pNIPAM strength = 80% cyanoacrylate).
6. Scleral patch adhesion performance in IOP test currently does not meet performance criteria (it is allowing unacceptable amount of saline leak from incisions during in vitro, cadaveric porcine eye test).
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8. Preparing for animal studies in year two.

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Appendices.

Appendix A. Linear Pull Test Protocol

TATRC Vision Research Program:

TATRC Project: Uniaxial Adhesion Testing of Scleral Patches.

PI: M. Humayun

Date	Version	Approved
01May 2013	20130501-01	

Title: Uniaxial Adhesion Testing of Scleral Patches.

Purpose:

The purpose of this procedure is to provide a protocol for: 1) measuring the adhesion strength of adhesive polymeric material to dissected scleral tissue, and 2) comparing the performance results between tested samples. The goal of this test is to identify an adhesive polymer formulation that provides adhesive strength equivalent to cyanoacrylate, a commonly used bio-adhesive glue.

Scope:

This test will be conducted by researchers that are part of the TATRC Thermo-responsive Adhesive for Ocular Trauma study team. This includes members of the Humayun research group, the Thompson lab and the Gupta group, from USC.

This test protocol is limited to polymeric adhesive samples for the TATRC Thermo-responsive Adhesive for Ocular Trauma study. This includes polymeric samples containing any of the following: pNIPAM, cyanoacrylate, n-tert butylacrylamide, copolymers of these materials, other polymeric materials known to exhibit adhesive properties.

Responsibilities:

The following responsibilities are defined for this test protocol:

Tissue Dissections: Scleral tissue will be harvested from fresh (<48hrs from euthanization and enucleation), cadaveric porcine eyes. 1cm x 1cm square patches of scleral tissue will be dissected from the eye using surgical scalpel and tweezers.

Test Setup: Tissue and test samples will be mounted to the uniaxial tester. The uniaxial tension tester will need to be reset, loaded with the dissected tissue and test sample. Controlling computer must be reset for new test. A test form should be printed for a written recording of the test.

Pull Test: Running of the measurement test.

Data Recording: The measurement data should be saved to electronic file on the computer. Manual entry of the key values should be completed on the SOP form and added to the investigation folder.

Data Analysis: Electronic data processing of measurement data files. Analysis of test results independently and compared against other test results.

Definitions:

Please see original project proposal for definitions.

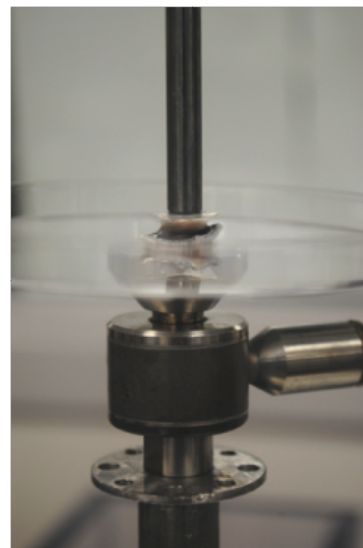


Figure 1. Photograph of uniaxial test system testing area; Load cell located at bottom, with clear, plastic petri dish mounted on top of it; cylindrical rod actuator rod located overtop and holding test samples and tissue between it and petri dish.

Materials & Equipment:

- Bose Uniaxial tension tester system with computer controller
- Cadaveric Porcine Eye
- Surgical scalpel/blade
- SS forceps (surgical)
- Petri dish/beaker for temporary tissue storage
- Thermometer, thermocouple or other temperature reading system
- Electrical heater
- Test Sample
- Timer/stopwatch
- Pipette to add/remove saline to petri dish

Procedures:

Scleral Dissection of Porcine Eye

1. Fill a glass container, e.g. petri dish, or wide beaker with at least 1cm of buffered saline.
2. Unpackage porcine eyes from delivery package. Porcine eyes should be used within 48 hours of delivery to ensure more accurate measurements.
3. Inspect the porcine eye for any signs of decomposition or poor tissue condition.
4. Using a surgical scalpel, dissect a large patch of scleral tissue from the area immediately peripheral to the iris. Large patch should be at least (1.5cm x 1.5cm). size can be larger and does not need to be square.
5. Once separated from the eye, trim the scleral patch into (1cm x 1cm) square patches.
6. Transfer the scleral patches to buffered saline for temporary storage.

Test Setup Mounting

1. Confirm that the 50g load cell is setup in uniaxial tester. If not, replace the load cell in the system with the 50g load cell.
2. Mount a plastic petri dish to the load cell metal flange with cyanoacrylate and allow 5 minutes to dry.
3. Stack 2-3 paper towels (stretched out) next to load cell for drying tissue.
4. Using forceps, transfer both of the scleral patches from the bath to the paper towel to dry. Pat both surfaces of both samples of the patches dry.
5. Identify the internal surface of the sclera on both samples and place this side up, i.e. put the exterior surface of the sclera facing down on the paper towel.
6. Setup the heating system so as to provide heat to the testing cell. Adjust temperature to meet desired temperature.
7. Add saline to petri dish up to height of tissues.

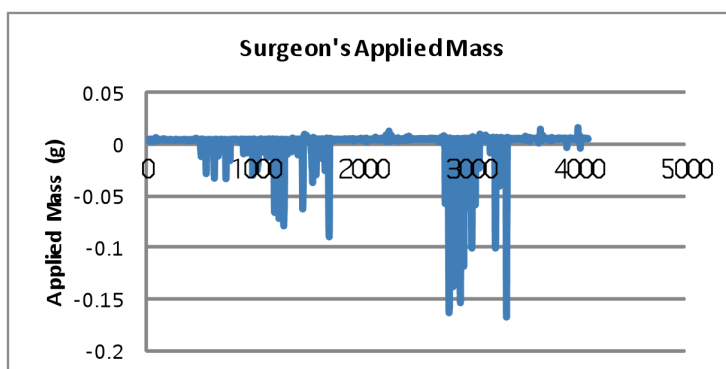


Figure 2. (left) Recorded force vs. time measurements used to determine application force to initiate adhesion of pNIPAM. Force was generated by surgeon applying pressure to pressure sensor, using tactile feedback to determine a clinically safe and acceptable pressure to apply to the sclera.

Test Procedure

1. Tests will be conducted at two different temperatures: 27°C (Room temperature) and 37°C (physiological temperature). Three measurements will be performed on each test sample at each temperature.
2. Set file name. Use appropriate filename that may include unique identifiers, e.g. date, material, test number, temperature. etc.
3. Pre-Loading Samples. Bring actuator down until the two scleral pieces come into contact with test sample between them. Reading the load cell's applied pressure, continue to lower the actuator arm until the pressure reads 15g of pressure.
4. Stop actuator at 15g of pressure and begin timing applied pressure. Allow pressure to be applied for 2 minutes before initiating pull test (next step).
5. At 2 minutes, begin linear pull test recording.
6. Pull test recording will record force vs. time. Force will rise until maximum adhesion is breached and then a sharp drop in force will be observed. at this stage the critical portion of the recording has been completed. The test can be ended after the force reaches a new baseline.

Data Recording:

1. Uniaxial Test Form should be filled out for each procedure (See Attachements).
2. Electronic file of measurement should be filed to study electronic folder.
3. Analysis data should recorded to performance data spreadsheet.

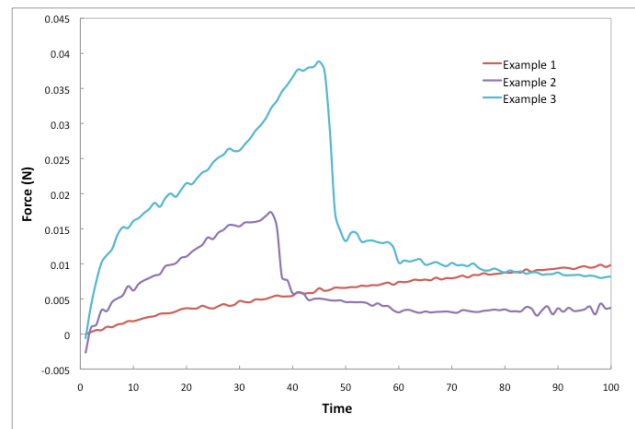


Figure. Example of isolated Force vs. time curve.

Data Analysis.

1. Maximum adhesion force to be identified and reported in mN units.
2. Curve profile through pull region may be isolated and used for comparison against other pull tests.

TATRC Vision Research Program:

TATRC Project:
In Vitro IntraOcular Pressure Adhesion Testing of Scleral Patches.

PI: M. Humayun

Date	Version	Approved
01May 2013	20130501-01	

Title: In Vitro IntraOcular Pressure Adhesion Testing of Scleral Patches.

Purpose:

The purpose of this procedure is to provide a protocol for: 1) measuring the adhesion strength of adhesive polymeric material to dissected scleral tissue in a cadaveric tissue model of the eye and intraocular pressure.

Scope:

This test will be conducted by researchers that are part of the TATRC Thermo-responsive Adhesive for Ocular Trauma study team. This includes members of the Humayun research group, the Thompson lab and the Gupta group, from USC.

This test protocol is limited to polymeric adhesive samples for the TATRC Thermo-responsive Adhesive for Ocular Trauma study. This includes polymeric samples containing any of the following: pNIPAM, cyanoacrylate, n-tert butylacrylamide, co-polymers of these materials, other polymeric materials known to exhibit adhesive properties.

Responsibilities:

The following responsibilities are defined for this test protocol:

Ocular Tissue: Scleral tissue will be harvested from fresh (<48hrs from euthanization and enucleation), cadaveric porcine eyes. Whole eyes will be used for this study. A partial vitrectomy will be performed to allow saline infusion, and to more accurately mimic the surgical conditions encountered in the clinic, in vivo.

Test Setup: The porcine eye will be mounted into a fixation stage (in the ocular orbit of a styrofoam model of the head). surgical microscopes will be used. An Alcon, Inc., Constellation Vitrectomy surgical system will be used. A heat lamp will be used to regulate temperature at the surface.

Cannulation, vitrectomy, penetration incisions suturing and patch placement were performed by an ocular surgeon.

Pressure Test: Running of the adhesion test.

Data Recording: The measurement data should be saved to electronic file on the computer. Manual entry of the key values should be completed on the SOP form and added to the investigation folder.

Data Analysis: Electronic data processing of measurement data files. Analysis of test results independently and compared against other test results.

Definitions:

Please see original project proposal for definitions.

Materials & Equipment:

- Alcon Constellation Vitrectomy System with trocars and supplies
- Heat Lamp
- Styrofoam head for ocular mount
- SS forceps (surgical)
- Petri dish/beaker for temporary tissue storage
- Ocular sutures
- Thermometer, thermocouple or other temperature reading system
- Test Sample
- Infusion cannula
- saline solution

Procedures:**Setup of Porcine Eye**

1. Remove porcine eye from packaging. With fixation pins, position and fix eye into orbit of styrofoam head mount.
2. Prep eye for and perform partial pars plana vitrectomy. Region of vitrectomy removal should be in the vicinity where perforation will be created.
3. Constellation infusion should be Measure and note baseline pressure and infusion flow of saline. Note - saline should be heated near infusion cannula to help with temperature properties of patch.

Test Procedure (next page)

1. Tests will be conducted at 37°C (physiological temperature).
2. Set file name. Use appropriate filename that may include unique identifiers, e.g. date, material, test number, temperature. etc.
3. Four measurements will be taken for each sample. One at baseline, one when the original incision is created, one when the suture has been placed and one when the patch is applied. Note: the first three measurements may be recorded once for a series all performed on the same eye. This will prevent excessive damage to the ocular tissue.
4. Once baseline pressure has been recored and infusion is confirmed to be setup with pressure settings fixed, create a small, straight incision through the scleral surface into the vitreous, approximately 2-4mm in length.
5. Measure and record the incision length. Measure and record the saline infusion flow rate once stabilized.
6. Using sutures, begin to close incision using standard suturing procedure for scleral lacerations.
7. Measure and record the saline infusion flow rate once stabilized.
8. Prepare patch to be used for closure.
9. Removing sutures from penetration site, apply patch to surface using acceptable pressure to hold in place. Use heat lamp exposure to provide additional heating if necessary. Note time used to fixate patch to eye.
10. Measure and record the saline infusion flow rate once stabilized.
11. Take notes or photographs regarding results. Measurement is over. Carefully remove patch.

Data Recording:

Data should be manually recorded to test protocol form.

Supporting Data:

The following is a catalog of additional studies performed in this quarter that investigated the effect of different PNIPAM preparations (e.g. cross-linking, dehydration, etc.) on adhesion force to conjunctiva and sclera.

Test Series A1. Control Study: Characterization of Tissue-Tissue Adhesion/Interaction

Key Findings/Conclusions:	Lower baseline for adhesion force measurements should be between 15 mN and 50 mN. PNIPAM should perform greater than 50mN
Scope:	The goal of this experiment is to measure the natural adhesion between tissues, to set a lower baseline for adhesion performance. Two pieces of dissected tissue will be pressed against each other <u>using no adhesive</u> between them. Pull testing will then be performed.
Test samples:	Two setups were tested. (Sclera)----(Sclera) and (Conjunctiva)----(Conjunctiva)
Application Protocol:	• 1cm x 1cm ocular tissue samples fixed to load cell and actuator arm • Arm moved down to make two tissue pieces contact each other • 5g or 15g pressure applied for 2 minutes, T = 38°C • Pull test initiated and F vs. time measured to maximum/failure.
Measure:	• Max force of Adhesion. • Number samples tested (n=2 for each) • Compare parylene-pNIPAM-sclera vs. parylene-PNIPAM-conjunctiva
Results/Observations:	There is a small adhesion interaction between the two tissues. However this interaction is 10^1 smaller than the adhesion strength exhibited by cyanoacrylate.

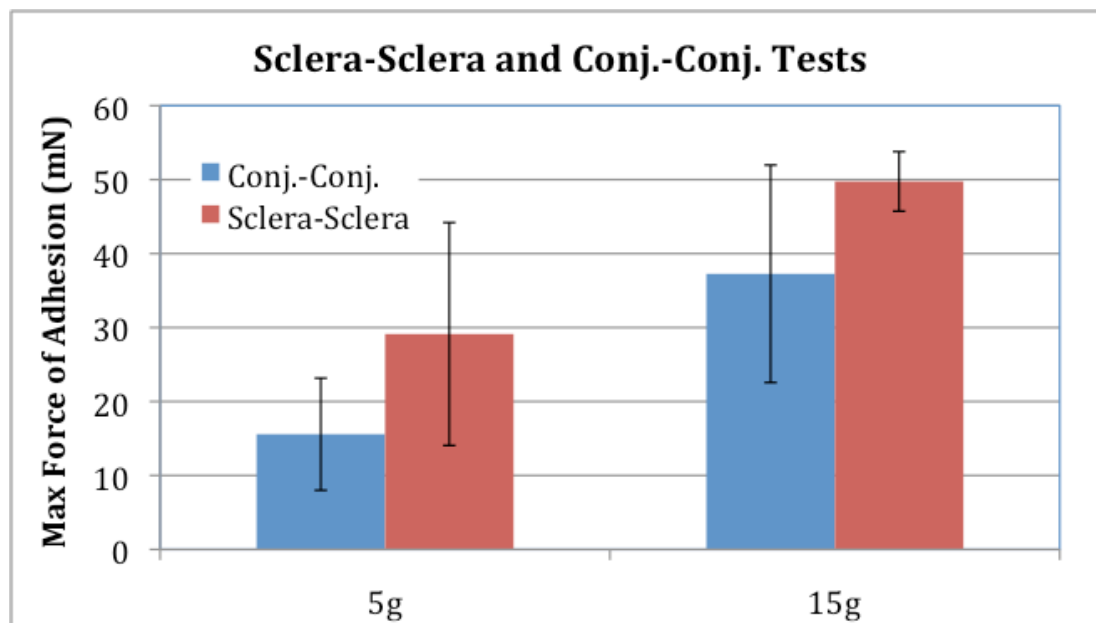


Figure A1. Second control series performed to measure tissue-to-tissue adhesion properties. This was used to set a minimum performance baseline.

Test Series A2. Adhesion Testing of first crosslinked (XL1) PNIPAM-Parylene patches

Key Findings/Conclusions:	The fabrication process used to produce this PNIPAM did not produce adhesion results meeting design criteria.
Scope:	Previous results suggested that crosslinking PNIPAM may improve adhesion performance. Here we characterize a new series of ATRP cross-linked PNIPAM on parylene.
Sample Tested:	• 100 micron ATRP-synthesized & crosslinked PNIPAM-Parylene samples
Control:	• Parylene patches with no PNIPAM (control, comparison)
Application Protocol:	• Patches pressed against tissue for t = 2min using either Force = 5g, 15g of pressure. • 1cm x 1cm patches used for all measurements.
Measure:	Compare effect of 5g vs. 15g application pressure at T = 15°C, 38°C
Results/Observations:	• Data reported in figure as Force (N) vs. temperature • The force in parylene (control, n=1) and PNIPAM/parylene (n=4) presented no significant difference; • Measured forces are equivalent to tissue-tissue adhesion seen in Test Series 2. • No significant adhesion performance measured with these samples.

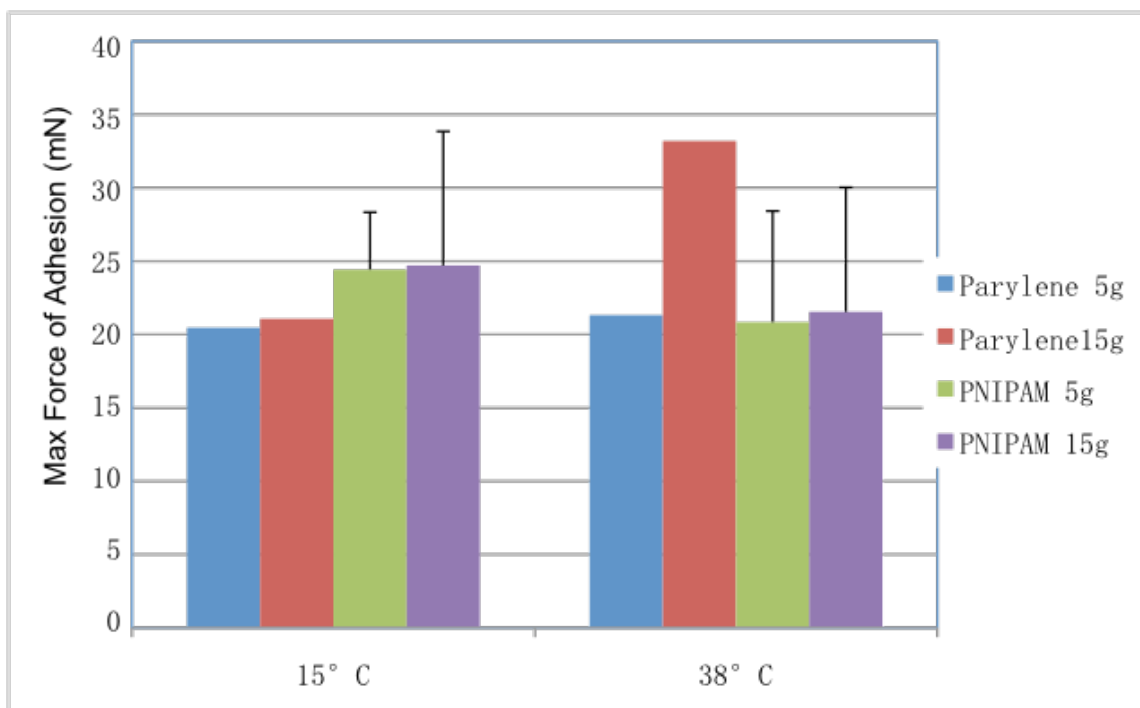


Figure A2. Adhesion force data on first crosslinked series of PNIPAM on parylene tested.

Test Series 3a: Adhesion Testing of second crosslinked (XL2) PNIPAM patches

Key Findings/Conclusions:	The crosslinking method used to produce this series did not yield improved adhesion. Still below performance requirements.
Scope:	Thompson and Gupta groups has prepared a new series of PNIPAM-parylene patches using a different crosslinking protocol. Here we test adhesion forces.
Sample Tested:	2 nd series of crosslinked PNIPAM-on-Parylene patches (n=4)
Control:	Compare against previous series (X-ATRP-1 and Parylene-ONLY patches)
Application Protocol:	Patches pressed against tissue for t = 2min using either Force = 5g, 15g of pressure.
Measure:	Compare effect of 5g vs. 15g application pressure at T = 18°C, 38°C
Results/Observations:	- No improvement in adhesion properties was observed in these samples. - Adhesion still < 50 mN.

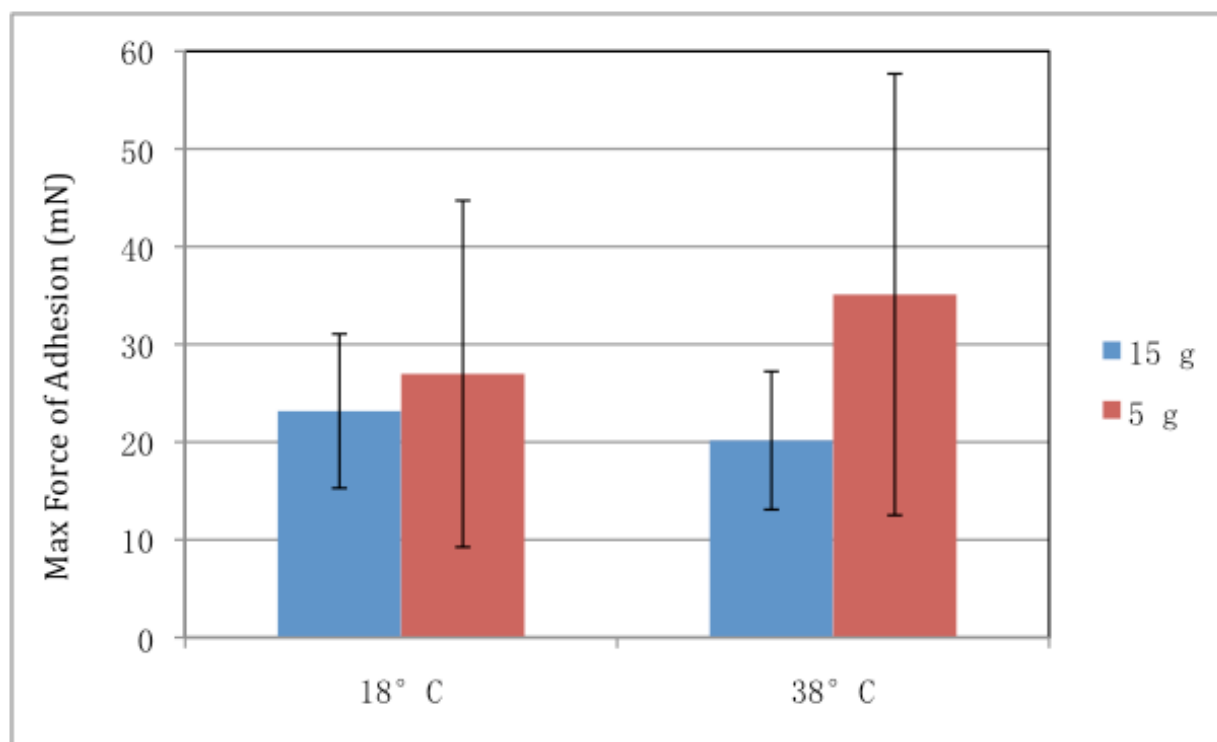
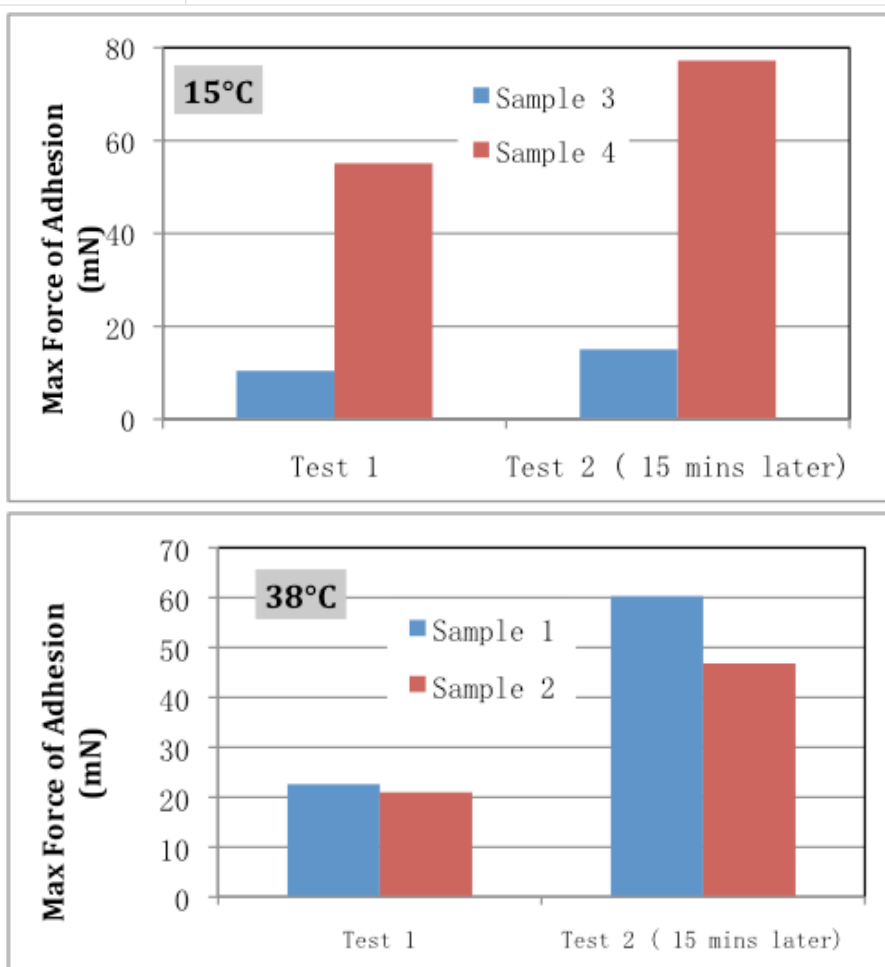


Figure A3. Adhesion testing of second series of cross-linked PNIPAM on parylene test samples.

Test Series A4: Characterization of Hydration Effect on adhesion.

Key Findings/Conclusions:	• No improvement in adhesion properties was observed in these samples.
Scope:	Anecdotal observations suggest that dehydration of samples may improve adhesion. This experiment was designed to evaluate this further.
Sample Tested:	Cross-linked PNIPAM-on-Parylene Series 2 (XL2)
Application Protocol:	• PNIPAM-parylene on ocular tissue • Patches pressed against tissue for $t = 2\text{min}$ using either Force = 5g, 15g of pressure. $T = 15^\circ\text{C}$ and 38°C • Samples were tested once, then 15 minutes passed then tested again. • Between test cycles, ocular tissue showed some drying out.
Measure:	• 15g application pressure • Performed once, then repeated again after 15 minutes resting. • Tests performed at $T = 18^\circ\text{C}$, 38°C
Results/Observations:	• First test on all samples significantly lower ($F = 20\text{mN}$) • Second test closer to 50 mN (closer to baseline goals) • Adhesion still less than 100 mN.



Figures A4 & A5. Evaluation of the effect of dehydration of ocular tissue on the adhesion response of PNIPAM patches.