REVERSIBLE THERMOSENSITIVE GLUE FOR RETINAL IMPLANTS

MURAT TUNC, MD,*† XUANHONG CHENG, MD,‡ BUDDY D. RATNER, MD,§ ELLIS MENG, PHD,¶ MARK HUMAYUN, MD*

Purpose: To determine in vitro effects of a plasma polymerized *N*-isopropyl acrylamide (pNIPAM) coating for thermally controllable adhesion to retinal tissue.

Methods: Polyimide (50 μm), parylene C [poly(monochloro-*p*-xylylene)] (20 μm), and poly(dimethyl siloxane) (PDMS) (200 μm) coated with pNIPAM were used as implant materials to test retinal adhesion in enucleated pig eyes. Following preparation of the implant materials (n = 5) and retina, the authors held the implants over the retinal tissue at 22°C and gradually increased the temperature of the water bath within 15 minutes. While increasing the temperature the authors monitored the adhesion with the retina and pNIPAM coated implant. The authors measured the adhesive force by a traction test using a suture attached to the implant and a strain gauge. Then the authors checked the reversibility of the adhesion by lowering the temperature of the water bath.

Results: There was no retinal adhesion at room temperature (22°C). The adhesion developed strongly within 60 seconds after reaching the critical temperature (≥32°C). This adhesion was persistent when the authors applied tractional forces of 98 mN and 148 mN between 32 and 38°C. When the authors lowered the temperature back to 22°C by irrigation with cold BSS, the implants detached from the retinal surface without using any tractional force.

Conclusion: pNIPAM provides effective in vitro retinal adhesion between 32 and 38°C and this adhesion is completely reversible by lowering the temperature of the physiologic medium.

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Building reliable interfaces between biologic and engineered systems is one of the great challenges in biomimetic applications and for drug delivery purposes. A safe and effective adhesive can be very useful to implant a biomimetic microelectronic device inside the eye. Several adhesives such as hydrogels, fibrin sealants, and photocurable glues have been tested in previous studies for this purpose.[1–3] These reports showed limitations in previously tested adhesives such as inflammation, toxicity, insufficient adhesive strength, irreversibility, and deformation of the ocular tissue.[1–6]

Polymeric systems that may modify their adhesive properties in response to changes in the physical and chemical characteristics of the physiologic medium are promising candidates to achieve reversible tissue adhesion. Several groups have explored the use of dynamic stimulus-responsive surface chemistries for cell patterning.[1,7–9] Thermoactive,[7] electrical-active,[8] and photoactive[1,9] chemistries have been defined for cellular adhesion. In general, all of these chemistries operate under the same principle: these substances can be switched from a state that prevents cellular attachment to a state that promotes it.

From *Doheny Eye Institute, ¶Biomedical Engineering, University of Southern California, Los Angeles; †Ophthalmology, Duzce University Medical School, Turkey; and ‡Bioengineering Department and §Department of Bioengineering and Department of Chemistry, University of Washington Engineered Biomaterials, Seattle.

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Reprint requests: Murat Tunc, MD, Inkilap Sokak 19-1, Kizilay, Ankara 06420, Turkey; e-mail: mtunc@isbank.net.tr

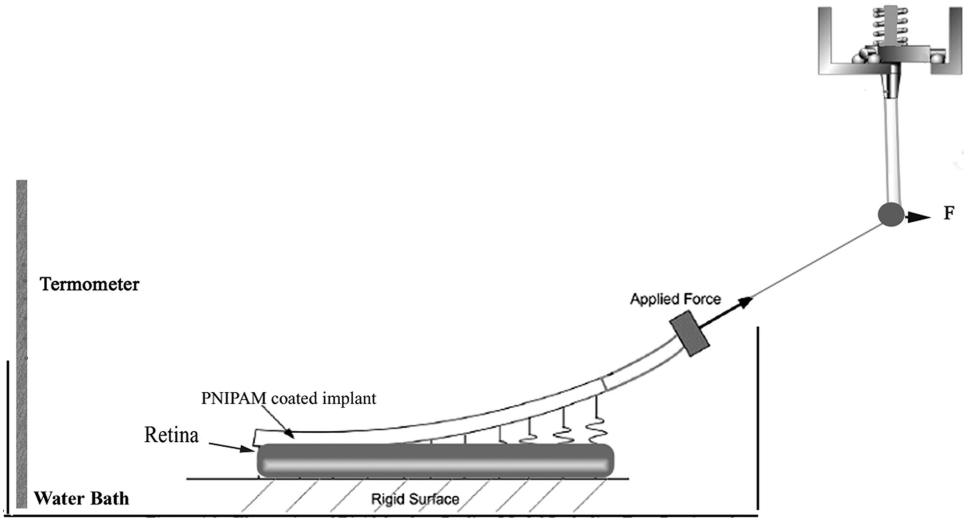

Fig. 1. Schematic diagram demonstrating the experimental design. (F = tractional force.)

In this study, we planned to demonstrate in vitro retinal tissue adhesion properties of a thermoresponsive adhesive: polymerized *N*-isopropyl acrylamide (pNIPAM).

Methods

We used three types of base materials: polyimide, parylene C [poly(monochloro-*p*-xylylene)], and poly-(dimethyl siloxane) (PDMS) for retinal implantation. These are inert materials that are commonly used for insulation of the epiretinal electrode for epiretinal stimulation. Polyimide was prepared as 50 μm thick films, parylene as 20 μm thick films, and PDMS was prepared at 200 μm in thickness at the Biomedical Engineering Department of the University of Southern California.

Coating of the base materials with a polymerized form of NIPAM was performed by plasma deposition as described elsewhere.[10] *N*-isopropyl acrylamide (NIPAM) (97%) was purchased from Aldrich (Milwaukee, WI) and following plasma deposition, the exposed surfaces of the implant materials (polyimide, parylene, and PDMS) were coated by pNIPAM. The pNIPAM-grafted surfaces were rinsed three times with cold deionized water to remove uncrosslinked molecules before use.

Enucleated pig eyes were transferred to the laboratory in cooled oxygenated 0.01 moles/L phosphate-buffered saline (PBS, pH 7.4), and the cornea, lens, and vitreous were totally removed. Vertical relaxing incisions were performed through the eyecup from the periphery towards the optic disk, leaving the retina intact at the posterior pole. The retina was stabilized over a soft plastic sheet by pinning with four 25-Gauge needles, to keep the retina flat and facing upward over the scleral surface. This preparation was irrigated by phosphate buffered isotonic ringer lactate solution in a water bath.

We used a thermostatic heater-controlled water bath to adjust the temperature of the retinal tissue. We checked the tissue temperature continuously during the experiment. pNIPAM coated materials, polyimide, parylene, and PDMS were cut into 3 $\times$ 2 mm pieces. Five pieces were prepared for each material. A 7/0 suture was passed through one corner of the polyimide and parylene implants to apply tractional force (peel test) controlled by a strain gauge mechanism to measure the adhesive force (Figure 1). As PDMS was too fragile to apply traction using the suture method, we only used vitreoretinal microforceps to check the retinal adhesion.

Five pre-prepared pNIPAM-coated implants for each material and five controls without pNIPAM coating were used in our experiments. Before retinal implantation, the water bath temperature was set at 22°C. We then gradually increased the temperature of the water bath to 38°C within 15 minutes. During the heating period we held the implant over the retina with vitreoretinal forceps. We checked the retinal adhesion between the pNIPAM coated materials and controls continuously until we reached body temperature (37°C). Where adhesion was observed, we measured the adhesive force by a strain gauge (Somfy-Tec-France) attached to the suture. Then we lowered the temperature back to 22°C to test whether the adhesion is reversible.

Results

At room temperature (22°C) there was no retinal adhesion in any of the pNIPAM-coated materials

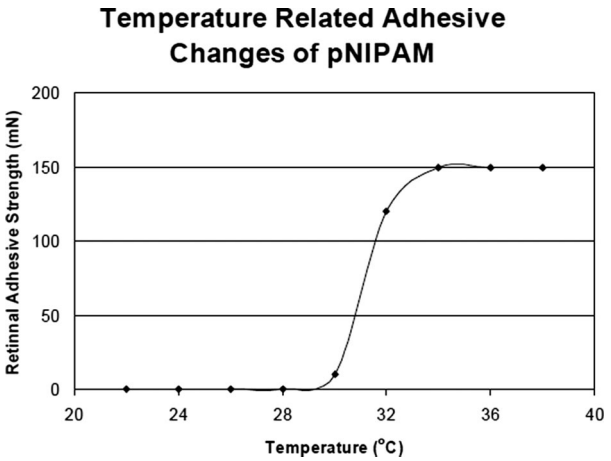

Fig. 2. Temperature-related changes in adhesive properties of polymerized *N*-isopropyl acrylamide (pNIPAM) coated implants.

(polyimide, parylene, and PDMS) and controls. Retinal adhesion developed in all pNIPAM coated test materials (polyimide, parylene, and PDMS) when the temperature reached 32°C. The adhesion developed strongly within 60 seconds after reaching the critical temperature (≥32°C). This adhesion was persistent between 32 and 38°C (Figure 2). We performed a pull test by a suture attached to one corner of the polyimide and parylene implants. Stable forces of 98 mN and 148 mN were applied for 5 seconds to pull these implants away from the retinal surface (Figure 3). The adhesion was stable during this test for both materials. We increased the tractional force up to 250 mN in two cases for each tested material and the retina tore or detached following increased peeling force.

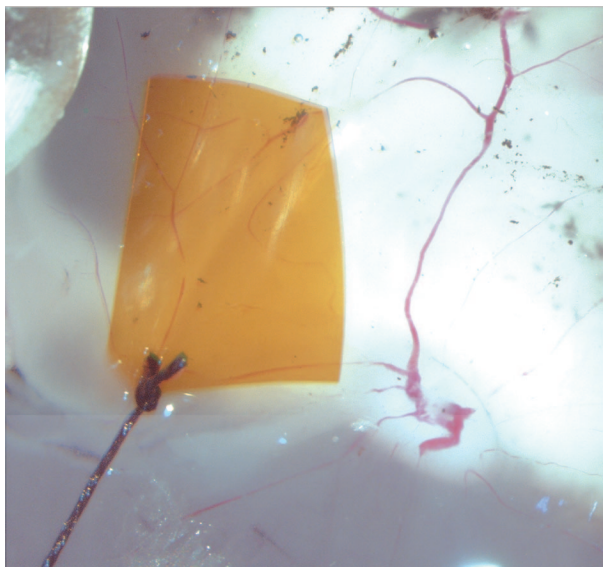

Fig. 3. Adhesion between the retina and pNIPAM coated implant during a peel test when we increased the temperature to body temperature. Retinal adhesion continues when we increase the pulling force.

In PDMS, we observed strong adhesion between 32 and 38°C and we were not able to detach the implant from the retinal surface with vitreoretinal forceps without detaching the retina from the scleral bed.

In the remaining three samples we lowered the temperature back to 22°C by irrigation with cold BSS. When we reached temperatures below 31°C all attached implants started to detach from the retinal surface spontaneously without using tractional force and they detached from the retinal surface completely within 2 minutes when we reached 22°C. This showed that the retinal adhesion of pNIPAM coated implants was reversible. There was no retinal adhesion in any of the controls without pNIPAM coating between 22°C and 38°C.

Discussion

A thermosensitive reversible adhesive could have many applications in ophthalmology such as in posterior segment surgery, implantation of biomimetic microelectronic devices, and ocular drug delivery. In addition, many other sites in the body could benefit from a reversible bioadhesive strategy for localized drug delivery, surgical repair, or the attachment of prosthetic devices. Previous studies showed that pNIPAM had a lower critical solution temperature of 31°C in an aqueous environment.[11,12] This means that the thermo-reversible hydrogel exhibits decreased solubility or swelling in water as the temperature is increased, due to a phase transformation at the lower critical solution temperature.[11,12] In this manner, pNIPAM can be switched from a state that prevents cellular attachment to a state that promotes the cellular attachment, merely by changing the temperature of the surface.[11] As this pNIPAM coating thermal transition is between room temperature and body temperature, the sharp property change and the ability to immobilize the polymer onto a solid support should be categorized as an enabling technology that can facilitate applications that were previously difficult or impossible.

Parylene, polyimide, and PDMS are inert materials that are commonly used for insulation of an epiretinal electrode for retinal stimulation. In our experiments pNIPAM coated implants showed strong adhesion with the retinal tissue in vitro above the transition temperature. There is no standardized method to test in vitro adhesive power of a material with the retina but when compared with previously tested adhesives, the adhesive strength of pNIPAM with the retinal tissue seems to be superior to cyanoacrylate,[2] fibrin sealants,[1] and Cell-Tak[1] and similar to hydrogels (succinimidyl succinate polyethylene glycol [SS-PEG]

and succinimidyl propionate polyethylene glycol [SPA-PEG]).[1] Cell adhesion onto a material surface is controlled by complex combination of physicochemical interactions including hydrophobic, coulombic, and van der Waals forces between the cell membrane and the material surface and molecular interpenetrations of macromolecules. SPA-PEG hydrogels can make covalent links with the retina.[1] pNIPAM undergoes its phase transition by changes in hydrophobic interaction and the breaking of H-bonds. Also, spectroscopic data showed a change of the polymer backbone conformation.[13,14] Thus, the adhesive force between retina and pNIPAM may be associated with the soluble, freely coiling chains that have slightly interpenetrated into the outer molecular structure of the retina and then undergone thermally induced hydrophobic collapse (the change in backbone conformation) locking themselves within the outer zone of the retina.

The ideal adhesive for intraocular use should be nontoxic and biocompatible. Previous reports showed that hydrogels such as SS-PEG and styryl-polyethylene glycol (ST-PEG) were effective but short-lasting and SS-PEG was toxic to the retina.[1] Although N-isopropylacrylamide monomer is toxic to neural tissue, following plasma polymerization, the pNIPAM molecule is no longer toxic to neural tissue and commonly used in cell cultures and tissue cultures for its reversible cell adhesion properties.[11,15,16] Previous reports showed cells attached and detached from pNIPAM coated culture dishes did not exhibit morphologic changes.[11,15] pNIPAM has also been used in retinal pigment epithelial (RPE) cell cultures to provide RPE sheets for transplantation and RPE cells preserved their morphology with no signs of toxicity.[15] Interestingly, pNIPAM has also been used to stop bleeding in experimental liver injuries and no toxicity has been reported.[17] To our knowledge there is no previous report on in vivo effects of pNIPAM inside the eye. Our in vivo study is underway to examine the long-term in vivo effects of pNIPAM on retina.

In vitro studies in porcine cadaver eyes showed that most of the measured force during retinal surgical manipulations was below 67 mN in magnitude.[18] In our study, pNIPAM coated test materials (polyimide and parylene) were resistant to tractional forces of 98 mN and 148 mN. Additionally, the adhesive force between the retina and pNIPAM-coated PDMS was strong enough to resist the pulling force of a vitreoretinal forceps. In regards to an intraocular implant, the force from any device on the retina depends on the weight of the device in air and then adjusted for the buoyancy effects of the intraocular environment.

Our study showed that all pNIPAM-coated implants can be attached and detached from the retinal surface, simply by changing the temperature of the retinal tissue. This reversibility theoretically may provide the ability to remove an epiretinal stimulating electrode whenever necessary simply by using a cooled BSS in infusion, facilitating exchange with a newer design in the future. In addition to its reversibility, pNIPAM coated implants may also provide better apposition of the electrode with the retinal surface for effective stimulation.[19]

Our in vitro results suggest that with patterning, especially in conjunction with microfabrication, thermoreversible hydrogels may be helpful in implantation of nanotechnological systems inside the eye. This thermoresponsive smart polymer may also have other potential applications in ophthalmic surgery and drug delivery. Our in vivo studies will provide detailed information for possible clinical applications of pNIPAM as a thermosensitive reversible adhesive.

Key words: biomimetics, pNIPAM, retina, thermosensitive glue.

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