Reversible bi-directional bending of hydrogel-based bilayer actuators

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Abstract

Temperature and pH responsive semi-interpenetrating network (semi-IPN) hydrogel based bilayer actuators were fabricated by generating a poly (N-isopropylacrylamide) (pNIPAm)-based hydrogel in the presence of positively charged polyelectrolyte poly (diallyldimethylammonium chloride) (pDADMAC) on a layer of gold-coated polydimethylsiloxane (PDMS). The bilayers showed unique bidirectional bending behavior in response to solution temperature and pH, which is a result of the modulation of the hydrogel swelling state in response to solution temperature and pH changes. The behavior described here is vastly different than what is observed from bilayers composed of just pNIPAm. The direction and degree of bending of the device could be easily adjusted by tuning the composition of the hydrogel layer. We also showed that the bilayers could be used as stimulus-induced grippers and for controlled/triggered small molecule delivery, which can make the bilayers useful for various biomedical applications, among other things.

Abstract: Stimuli-responsive polymers; bilayer actuators; artificial muscles; drug delivery

1. Introduction

Stimuli responsive ("smart") polymer-based hydrogels are highly water swellable crosslinked polymer networks,[1] which undergo abrupt volume changes in response to changes in their environment, e.g., temperature, pH, ionic strength, exposure to specific small molecules and application of an electric field.[2, 3, 4, 5] Consequently, they play a very important role in many practical applications such as sensing,[6, 7] drug delivery,[8, 9] soft actuators and robotics,[4, 10] and artificial muscles.[11, 12] While traditional hydrogel networks are held together by covalent crosslinks, there are a class of hydrogels called interpenetrating polymer networks (IPN) that are formed by combining two polymers (at least one should be responsive) that have some physical interactions that hold the network together.[13] Additionally, there are semi-IPNs that are composed of a covalently crosslinked hydrogel infused with another linear polymer physically interpenetrated inside the network.[14] Compared with single network hydrogels, it is possible for the components of the IPNs to work together to yield materials with new behavior that is not expected from the responses of the individual components alone.[15] Most research on IPNs is focused on gaining a better understanding of their fundamental properties (e.g. their swelling/deswelling behavior),[16, 17] on their practical applications as biomaterials[18] and as novel sorbents for separations.[19] Here, for the first time we describe a novel semi-IPN hydrogel-based actuator composed of two layers. One layer is composed of a poly (N-isopropylacrylamide) (pNIPAm)-based hydrogel interpenetrated with linear poly (diallyldimethylammonium chloride) (pDADMAC); the other layer is polydimethylsiloxane (PDMS).

PNIPAm is one of the most well-known temperature responsive (thermoresponsive) polymers, which undergoes a coil-to-globule transition at temperatures above its lower critical solution temperature (LCST) of ~32 °C.[20] Like linear pNIPAm, pNIPAm-based hydrogels undergo a transition from a swollen to a collapsed state upon heating above pNIPAm's LCST. PNIPAM's thermoresponsivity has been exploited for a variety of applications, including cell culturing,[21] drug delivery,[22] and soft actuators.[23] Introducing ionic groups into the neutral pNIPAm network adds an additional osmotic pressure contribution to solvation state of the network due to the high concentration of mobile ions within the network as a consequence of the Donnan equilibrium.[24] This renders ionic hydrogel networks responsive to dual stimuli, i.e., responsive to temperature and pH/ionic strength. For example, a pH and temperature responsive network can be formed by copolymerizing a weak acid with NIPAm.[25] One of the most common weak acids used for this purpose is acrylic acid (AAc), which has a pKa of ~4.25. Therefore, AAc is deprotonated when the solution pH is higher than 4.25, which leads to a high network osmotic pressure, and a concomitant swelling of pNIPAm-co-AAc hydrogel network. Likewise, the network collapses when the AAc groups are protonated at low pH.

In this study we use pDADMAC as the interpenetrating species in the pNIPAm-based network. PDADMAC is a strong polycation that exhibits a permanent positive charge on its backbone. PDADMAC-based gels capable of absorbing several hundred times their own volume of water,[26] which makes them a good candidate for improving water swellability of hydrogel networks. For example, a series of IPN hydrogels were synthesized using pDADMAC and other components, such as chitosan,[27] and poly (vinyl alcohol),[28] and were shown to exhibit large-scale volume changes that were significantly greater than the pure hydrogels alone.

PNIPAm hydrogel-based bilayer actuators have been reported previously in the literature, and their actuation relies primarily on the temperature-induced volume change of a pNIPAm-based layer.[29, 30, 31, 32] However, these actuators are generally limited to one directional deformation, and exhibit low stiffness, which makes them too soft for many practical applications. In order to address the limitations of these traditional thermoresponsive bilayers, we fabricated semi-IPN pNIPAm hydrogel-based bilayers. These devices are composed of a PDMS layer, and a hydrogel layer formed from polymerization of a solution of NIPAm in the presence of linear pDADMAC. Due to the extremely high water retention of pDADMAC, this semi-IPN hydrogel layer swells to a large extent at low temperature; to a much greater extent than the hydrogel without pDADMAC. Additionally, the semi-IPN layer undergoes a large-scale volume change as a result of heating above the LCST of pNIPAm. By depositing this hydrogel layer on a sheet of polydimethylsiloxane (PDMS) with a relatively higher tensile strength, the overall stiffness of semi-IPN hydrogel layer is increased. The PDMS layer also serves as a non-water swellable layer, which is dissimilar to the water swellable pNIPAm-based hydrogel layer.

In this submission, we investigated the ability of the pNIPAm-based semi-IPN bilayers to bend in response to temperature changes. Furthermore, we show that by copolymerizing AAc into the hydrogel layer, the devices can bend in response to pH. It is important to note that the pNIPAm-based semi-IPNs generated here exhibit bi-directional bending, which is very different than devices composed of just pNIPAm-based hydrogel layers. By generating these devices, more advanced actuators can be generated that will lead to the development of more sophisticated biomimetic actuators, manipulators and small molecule release devices.

2. Results and discussion

2.1 Strategies for design and fabrication of bilayers

PNIPAm-based hydrogels have relatively low stiffness,[33] which are further softened by addition of components that render the networks more hydrophilic/swellable.[34] In this study we attached a soft, stimuli responsive pNIPAm-based hydrogel layer onto the surface of the more rigid PDMS; again, we note that PDMS is not swellable with water. The active hydrogel layer exhibits significant volume expansion/contraction by swelling/deswelling upon exposure to certain stimuli, while the volume of PDMS remains relatively constant. It is this differential swellability that generates the internal stress required for bi-directional bending of the generated bilayers.

The fabrication process of the hydrogel/PDMS bilayer is shown schematically in Figure 1. Specifically, a thin PDMS sheet (~ 1 mm) was coated with Au by thermal evaporation. Then, devices with a defined size and shape were cut out of the PDMS sheets. After modification of the Au layer with 1-propene-2-thiol (to allow the hydrogel layer to covalently attach to the surface), the aqueous pre-gel solution composed of NIPAm, AAc (if desired), N, N'-methylenebisacrylamide (BIS) as crosslinker, pDADMAC (one of the three different molecular weights investigated), and 2,2-diethoxyacetophenone (DEAP) as photoinitiator, was deposited on the Au-coated PDMS. To make a thin (relative to the PDMS layer thickness) and homogeneous hydrogel layer, a Teflon sheet (thin enough to allow light to penetrate and irradiate the pre-gel solution) was placed on top of the pre-gel solution, as shown in Figure 1b. Capillary forces and gravity allowed the Teflon to sandwich a layer of the pregel solution, and the thickness of the resultant hydrogel layer could be controlled by the amount of pre-gel solution used. The assembly was placed on a cold plate set to maintain its temperature at 10 °C while the device was exposed to UV light for 15 min to initiate polymerization. The polymerization was completed at low temperature to ensure that the hydrogel remained in its hydrated state during polymerization, which will allow for maximal swelling/deswelling. After polymerization, the Teflon was removed from the hydrogel surface leaving the hydrogel film intact and attached to the PDMS. The resultant bilayers were then soaked in DI water for several days to remove the unreacted monomers and the excess pDADMAC, which was not successfully trapped in the hydrogel network.

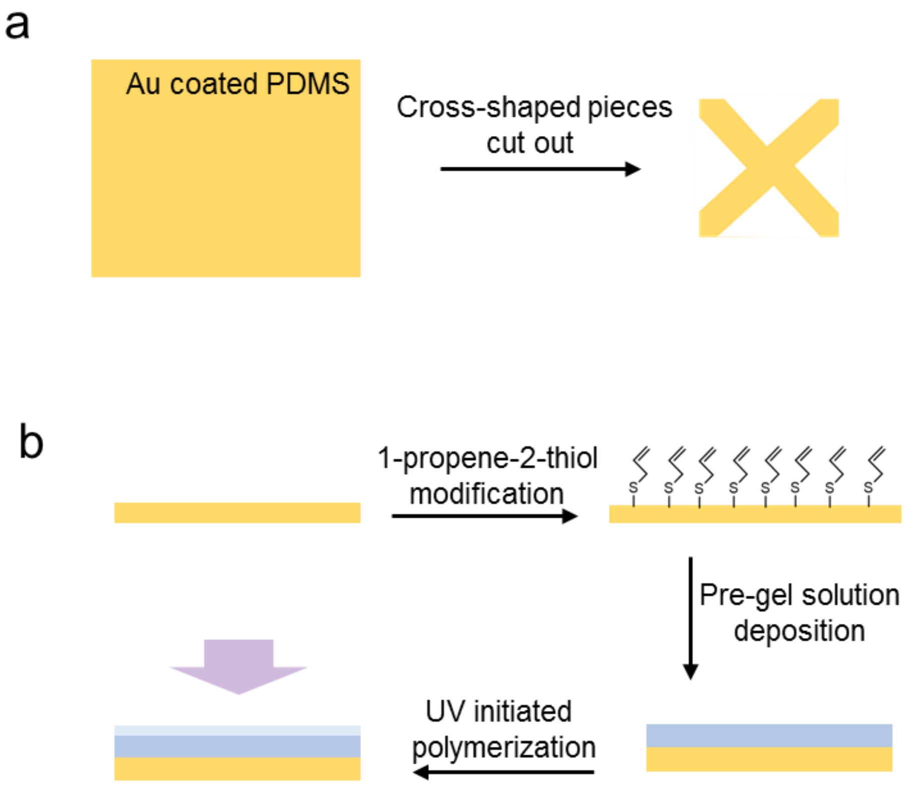

Figure 1 a) Schematic illustration of the fabrication process of cross-shaped PDMS substrates; b) the surface of the pieces were modified with 1-propene-2-thiol and hydrogel layers photopolymerized on top of PDMS layers by exposing the pregel solution (blue layer) to UV-light through a piece of Teflon (grey top layer). The Teflon was removed after formation of the hydrogel layer.

2.2 Thermoresponsive bi-directional self-bending bilayers

To investigate the thermoresponsivity of the generated bilayer system, we prepared pNIPAm hydrogel/PDMS and pNIPAm-pDADMAC semi-IPN hydrogel/PDMS bilayers. We used pDADMAC with three different molecular weights (100-200 kDa (low Mw), 200-350 kDa (medium Mw), and 400-500 kDa (high Mw)). To obtain hydrogel layers with the similar thicknesses, we deposited the same amount of pre-gel solution on the surface of PDMS layer, followed by the placement of Teflon sheet on top. The thickness of the thermoresponsive hydrogel layers were determined to be ~0.8 mm. In order to investigate the structure of the hydrogel/PDMS interface we obtained scanning electron microscopy (SEM) images of freeze-dried bilayers. As shown in Figure 2 the hydrogel layer is extremely porous, exhibiting micrometer-scale pores. The image clearly shows the crosslinked hydrogel network attached to the PDMS surface. Furthermore, the two layers do not separate even after many rounds of bending and unbending (data not shown).

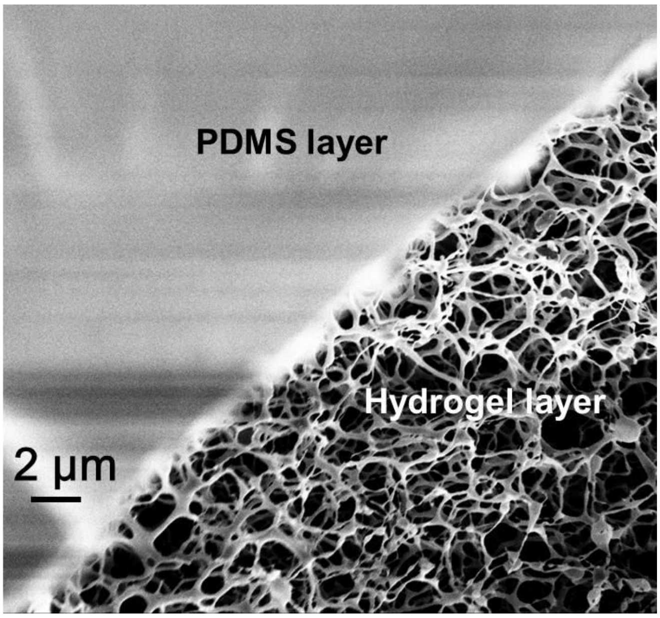

Figure 2. SEM image of the interface of the two layers: the porous side is the hydrogel layer, while the other side is the PDMS layer.

Next, the ability of the resultant devices to bend in response to temperature changes was evaluated. Figure 3a shows that the bilayer, with pDADMAC trapped in the pNIPAm hydrogel layer, is transparent and bends towards the PDMS layer into a spherical capsule at low temperature (25 °C) in DI water. The thickness of the gel was measured to be ~2.5 mm at these conditions. When the temperature was increased to 40 °C, the hydrogel layer became white as a result of the transition of the pNIPAm-based network from a swollen to deswollen state. The thickness of the gel was measured to be ~0.8 mm at these conditions. This leads to an opening of the capsule and subsequent bending of the bilayer toward the hydrogel layer. Based on the measured hydrogel layer thicknesses at the indicated conditions, we determined that the degree of hydrogel shrinking was ~77 % (v/v). The bilayers were able to completely bend back to the original spherical capsule conformation when the temperature was decreased to 25 °C. We point out that the bending processes were complete within ~10 mins. It is also worth mentioning that the hydrogels generated from the pDADMAC of three different molecular weights show very similar bending behavior in response to temperature changes. In contrast, as shown in Figure 3b, the bilayer system without pDADMAC failed to exhibit the same bi-directional bending behavior. At low temperature, in the absence of pDADMAC, the mechanical force generated from the volume expansion of the pNIPAm hydrogel layer by swelling was not strong enough to cause the bilayer to

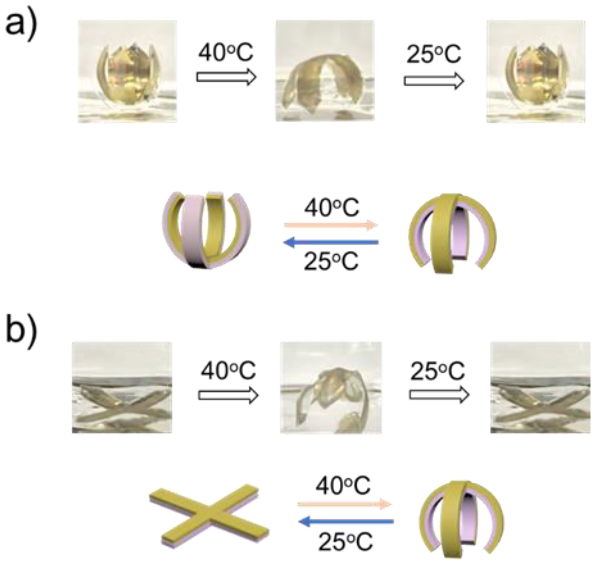

Figure 3. a) Actuation of the pNIPAm-pDADMAC hydrogel/PDMS bilayer system; b) actuation of the pNIPAm hydrogel/PDMS bilayer system. The schematics in both a and b are to clarify the bending process; the yellow side is the PDMS layer, while the grey side is the pNIPAm-based hydrogel layer.

bend. Although at high temperature, the force generated from the shrinking of the hydrogel network could still induce one directional bending of the bilayer system toward the hydrogel. In this case, the degree of hydrogel shrinking was ~60%. SEM images were also collected after temperature cycling to determine if the hydrogel structure was affected by the bending. The images in Figure 4 (a1, a2, a3) show the pNIPAm-pDADMAC hydrogel layer after the 1st, 2nd, and 3rd cycle of bending at 25 °C, while a1', a2', and a3' show the images of the same hydrogel network at 40 °C for the 1st, 2nd, 3rd time. For example, a1 shows the SEM image of the hydrogel initially at 25 °C, a2 is the image of hydrogel at 25 °C after its first heating to 40 °C (a1'), and so on. We point out that the hydrogel structure was locked in at the specific temperatures by freezing the bilayers "instantaneously" by dipping in liquid nitrogen and subsequent freeze-drying. Figure 4 (b1, b2, b3) show the SEM images of the pNIPAm hydrogels after the 1st, 2nd, and 3rd cycle of bending at 25 °C, while b1', b2', and b3' show the images of the same hydrogel network at 40 °C for the 1st, 2nd, 3rd time. In general, we can conclude that the pore sizes of the hydrogel layers (with/without pDADMAC) at 25 °C are much larger than at 40 °C. This is attributed to the deswelling of the hydrogels at elevated temperature. The contraction of the hydrogel layers explains the bending of devices with and without pDADMAC towards the hydrogel layer at high temperature. From careful examination of the images in Figure 4, we can also conclude that the hydrogels with pDADMAC are more porous than those without pDADMAC. We hypothesize that the enhanced porosity is a result of the charged quaternary ammonium groups on pDADMAC, which generates a high osmotic pressure in the network, and a high porosity. The high porosity leads to a high swellability for the pDADMAC-containing hydrogels at low temperature, which allows them to bend toward the PDMS. This is in contrast to the hydrogels without pDADMAC, which do not have sufficient porosity/swellability to allow the bilayers to bend toward the PDMS at low temperature. As can be seen from the images, the pores are not greatly affected by temperature cycling, which explains the reversibility of the temperature induced bending.

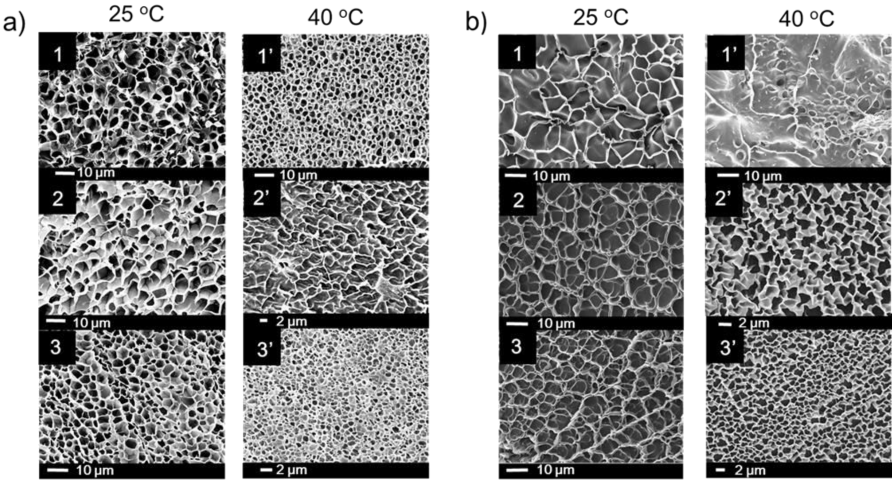

Figure 4. a1, a2, a3 are SEM images for the pNIPAm-pDADMAC hydrogel layer after the 1st, 2nd, and 3rd cycle of bending at 25 °C; a1', a2', and a3' are images of the same hydrogel network after the 1st, 2nd, 3rd cycle of bending at 40 °C; b1, b2, b3 are the SEM images for the pNIPAm hydrogel layer after the 1st, 2nd, and 3rd cycle of bending at 25 °C; b1', b2', and b3' are images of the same hydrogel network after the 1st, 2nd, 3rd cycle of bending at 40 °C;

As mentioned above, pNIPAm-based hydrogels are soft, and while they can exhibit temperature induced bending, they are not mechanically robust enough to do significant work. The devices here are much more mechanically robust than the hydrogels themselves as a result of the PDMS in the structure. Figure 5 shows the pNIPAm-pDADMAC hydrogel/PDMS bilayers being used as temperature activated grippers. When the temperature of the solution the bilayer is exposed to is decreased from 50 °C to 10 °C the bilayer bends towards the PDMS, allowing it to grip a plastic bead. The bilayer opens up and releases the bead when it is exposed to water at 50 °C. This cycling can be repeated multiple times. We point out that the gripping occurs at low temperature, which is quite different than most pNIPAm-based actuators, which are usually activated to grip objects above the LCST. Finally, since the bilayers grip the object on the PDMS side of the bilayer, it prevents the pNIPAm-based hydrogel layer from being damaged, improving the device lifetime. This is not the case for bilayers without pDADMAC, which only bend toward the hydrogel side of the bilayer at elevated temperature.

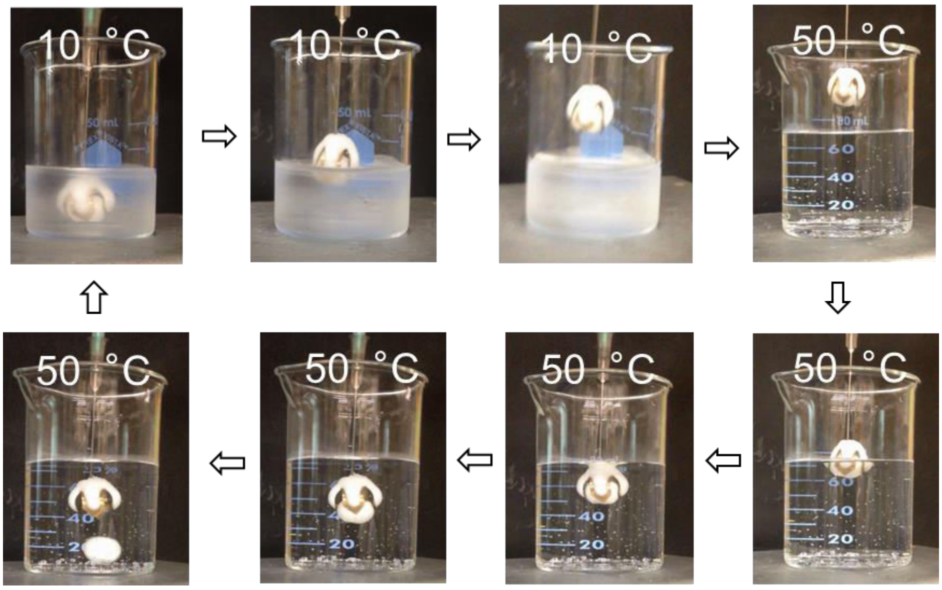

Figure 5. A bilayer is able to grip an object at 10 °C due to the hydrogel swelling (top, left). Note the beaker in the low temperature images appear cloudy due to water condensation on the cold glass. At elevated temperature (50 °C) the bilayers open up and release the object.

2.3 pH-responsive bi-directional self-bending bilayers

In order to render the bilayers pH responsive, we copolymerized AAc into the hydrogel layer and evaluated the resultant pH-dependent bending behavior. These bilayers were prepared with 15% AAc (mol %) with and without pDADMAC present (medium Mw). We chose this MW pDADMAC as we think it allows for maximal interaction between the linear pDADMAC and AAc in the hydrogel network. As can be seen in Figure 6a the bilayers generated with pDADMAC bend toward the hydrogel side of the actuator into a spherical capsule at pH 6.5 (AAc groups are deprotonated and negatively charged). Figure 6a1 shows a photograph of the bent bilayer, and Figure 6a2 shows the corresponding schematic diagram. When the solution pH was changed to 3 (AAc groups are protonated), the bilayer bends in the opposite direction, as shown in Figures 6a1' and 6a2'. This bi-directional bending can be repeated many times as the solution pH is switched between 6.5 and 3. In contrast, the bilayer system with only NIPAm and AAc in the hydrogel layer (i.e., no pDADMAC), bends toward the PDMS instead of into a capsule at pH 6.5, as shown in Figure 6b1 and Figure 6b2. When the pH is changed to 3, the capsule opens up to be approximately flat without bending in the other direction. We point out that all the above pH-dependent experiments were conducted at 25 °C.

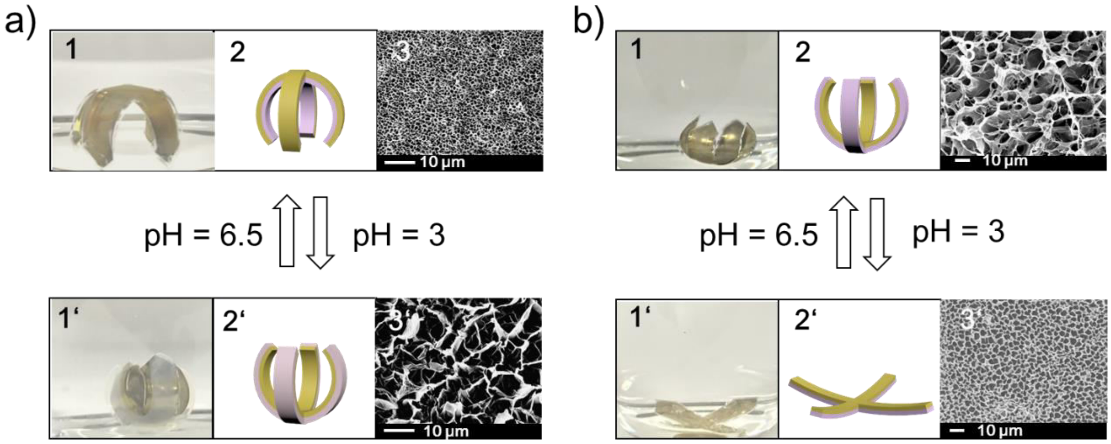

Figure 6. a1, a2, a3 are the photograph, schematic illustration, and SEM image, respectively, of the pNIPAm-AAc (15%)-pDADMAC hydrogel layer at pH 6.5; a1’, a2’, a3’ are images for the same hydrogel layer at pH 3.0; b1, b2, b3 are images for the pNIPAm-AAc (15%) hydrogel layer (no pDADMAC) at pH 6.5; b1’, b2’, b3’ are images for the same hydrogel layer at pH 3.0.

From an examination of bilayer microstructure in the SEM images of the pNIPAm-15% AAc-pDADMAC hydrogels in Figure 6a3 (pH =6.5) and Figure 6a3’ (pH = 3), we propose that the small sized pores in Figure 6a3 are a result of the strong electrostatic association of the negatively charged AAc (belongs to the chemically crosslinked hydrogel network) and the positively charged pDADMAC (belongs to the physically trapped polyelectrolyte chain) in the hydrogel network. This promotes the shrinking of the hydrogel at pH 6.5 and leads to the bending of the bilayer towards the hydrogel layer. On the other hand, AAc is neutralized at pH 3, which allows the pDADMAC to be released from interaction with AAc groups, and recover its ability to absorb a large amount of water. Consequently, the pore size of the hydrogel network at pH 3 (Figure 6a3') is much larger than at pH 6.5. The resulting high osmotic pressure promotes the bending of the bilayer towards the PDMS layer. In contrast, the pore size of the pNIPAm-15% AAc hydrogel at pH is 6.5 is relatively large due to the osmotic pressure generated by the existence of deprotonated AAc (shown in Figure 6b3), which leads to the bending of the bilayer towards the PDMS layer. On the other hand, at pH 3, the protonation of AAc leads to a decrease in pore size and shrinking of the hydrogel network (shown by Figure 6b3'), which allows the capsule to open up to be almost flat. In addition, we further incorporated pDADMAC with low Mw and high Mw in the pNIPAm-15% AAc hydrogel layer. It was observed that the bilayer with high Mw pDADMAC trapped in the hydrogel layer exhibits bending behavior that is similar to the bilayer with the medium Mw pDADMAC trapped in response to pH switching between 6.5 and 3.0. However, the bilayer with low Mw pDADMAC trapped shows a small degree of bending towards the PDMS layer at pH 6.5 (photographs are not shown here), which is different from the other two pDADMAC MWs. We hypothesize that this is a result of the low Mw pDADMAC being unable to span as much of the network compared to the medium and high Mw pDADMAC, thus the electrostatic interactions formed are not strong enough to induce the shrinking of the hydrogel network at pH 6.5. However, the bending behavior of these three types of bilayer systems is similar at pH 3. Likewise, at pH 3 the protonation of AAc frees pDADMAC from electrostatic interaction with the negative AAc charges, leading to a larger osmotic pressure in the hydrogel layer and its swelling. Thus the bilayers bend towards the PDMS layer.

2.4 Dual stimuli responsive self-bending cross-shaped bilayers

We also investigated the effect of dual-stimuli on the bending behavior of the bilayers, using the pNIPAm-15% AAc-pDADMAC (medium Mw) hydrogel layer as an example. Figure 7a1 and Figure 7a2 show photographs and schematic representations of the bilayer structures at pH of 6.5 and temperature of 25 °C. As we expected, the bilayer systems bends toward the hydrogel layer.

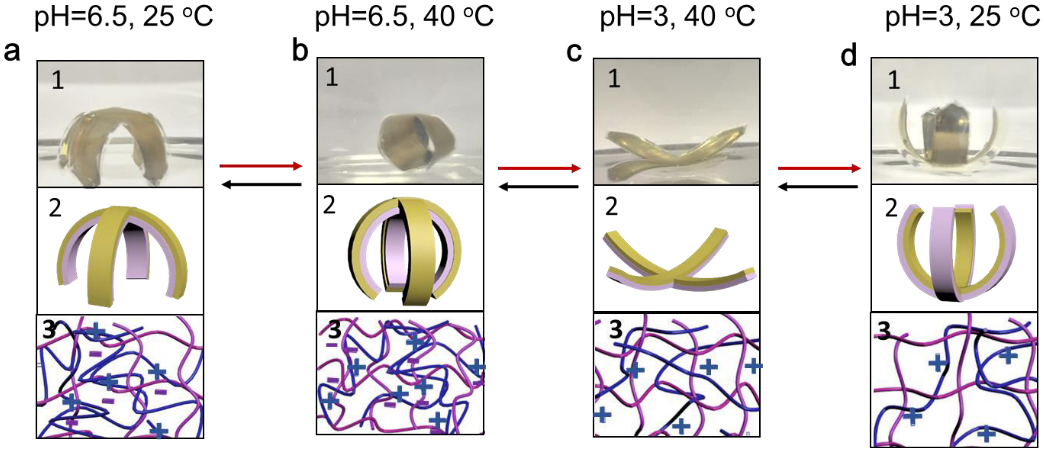

Figure 7. Bent bilayer structures, 1: photograph, 2: schematic diagram, 3: arrangement of polymer chains, at the indicated solution temperature and pH.

When the pH is maintained at pH 6.5 and temperature is increased to 40 ^{o}C, the bilayer bends further toward the hydrogel layer due to the pNIPAm thermoresponsivity leading to further contraction, as shown in Figure 7b1 and Figure 7b2. Furthermore, when the temperature was maintained at 40 ^{o}C and the pH changed to 3.0, we found that the completely bent structure opens up to become nearly flat. We proposed that the protonation of AAc allows the positively charged pDADMAC to absorb water and swell, even at elevated temperatures. However, the copolymerization of AAc with NIPAm in the hydrogel increases the LCST of pNIPAm, which maintains the pNIPAm hydrogel network at a relatively deswollen state compared with the hydrogel network without copolymerized AAc. When the temperature was lowered to 25 ^{o}C, while maintaining pH at 3.0, the bilayers bend towards the PDMS, due to the swelling of the doubly complexed hydrogel network. Figure 7a3, Figure 7b3, Figure 7c3 and Figure 7d3 show the corresponding hypothesized schematic diagrams of the internal structure of the bilayers at the respective conditions. The bending states of the bilayer systems can be manipulated by simply varying pH and temperature in a way that yields the desired structure. On the other hand, the bending states of the bilayer can also be considered as an indicator of the environmental conditions (pH and temperature).

2.5 Application: Small molecule release from pH-responsive bendable bilayers

Finally, we studied the ability of the semi-IPN hydrogel-based bilayers to release small molecules in a controlled and triggered fashion. Here, we describe polymeric actuators that actively grab objects and absorb small molecules at some specific conditions, and subsequently release both species in a pH-triggered fashion. We selected the small molecule tris(4-(dimethylamino)phenyl)methylium chloride (Crystal Violet, CV) to be released from the bilayer device, which is positively charged. The hydrogel layers we investigated were composed of pNIPAm-25% AAc (mol%) with and without low Mw pDADMAC. As mentioned above, pDADMAC (low Mw) in the hydrogel network is not able to form long-range strong electrostatic interactions with deprotonated AAc throughout the hydrogel network. It is then hypothesized that more resultant free ends of trapped polymer chains renders the interaction between pDADMAC (low Mw) and AAc less stable and more dynamic. Thus when CV molecules are introduced into the hydrogel networks, they are more competitive in binding with AAc in the hydrogel in the presence of low Mw pDADMAC compared with medium Mw species. This leads to enhanced CV loading efficiency of the bilayer (83% for pDADMAC (low Mw) versus 70% for pDADMAC (medium Mw)).

We point out that when loading CV in the bilayer system, the bilayer system with pDADMAC self-bends towards the PDMS layer and is capable of gripping an object (for instance, a polymeric bead), as shown in Figure 8b1 (the loading process is illustrated in detail in the experimental section). This serves as a proof-of-concept that our bilayers have the potential to grab large objects (e.g., abnormal tissues or cells), and release small molecules (drugs) locally. The competitive binding of loaded CV with AAc in the hydrogel renders pDADMAC free in the hydrogel, which leads to the self-bending of the bilayer at pH 6.5. The average loading efficiency of the bilayer with/without pDADMAC (low Mw) trapped in the hydrogel layer is 83% and 97% respectively. The release of CV molecules from both CV-loaded bilayers was evaluated by monitoring the absorbance at 590 nm over time. When the pH of solution was decreased to 3.0, the protonation of AAc leads to the release of CV out from the hydrogel layer due to the loss of electrostatic interactions between CV and AAc moieties. As indicated in Figure 8a, When the pH is adjusted to 3.0 the bilayers with pDADMAC clearly shows faster release rate of CV than the one without pDADMAC. This is due to the existence of positively charged pDADMAC (low Mw) with relatively high dynamics of moving inside the hydrogel network, which facilitates the expulsion of CV molecules out of the hydrogel network. As shown in Figure 8b2, Figure 8b3, and Figure 8b4 the bent capsule gradually opens up as more and more CV molecules are released.

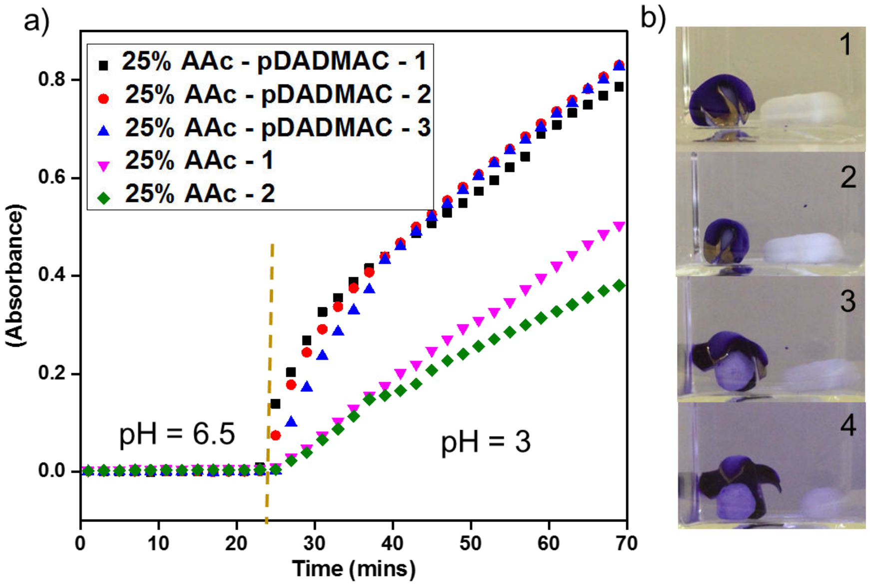

Figure 8. a) The release profiles of bilayers triggered by pH; b1, b2, b3, b4 represents the releasing of small molecules and as well as opening up of the bent bilayer device.

In addition, we show that CV molecules can be released from the bilayer system on demand by switching pH of the solution. To accomplish this, we first immersed the CV-loaded bilayers in pH 6.5 solution and monitored the release for a specific period of time. Then we lower the pH of the solution to 3.0 by adding hydrochloric acid (HCl) to induce the fast release of CV, followed by soaking the bilayer in pH 6.5 solution. This process was repeated and as shown in Figure 9, the on-off switching process for releasing can be repeated many times. In the meantime, the bilayers show reversible shape changes as well in responsive to pH changes.

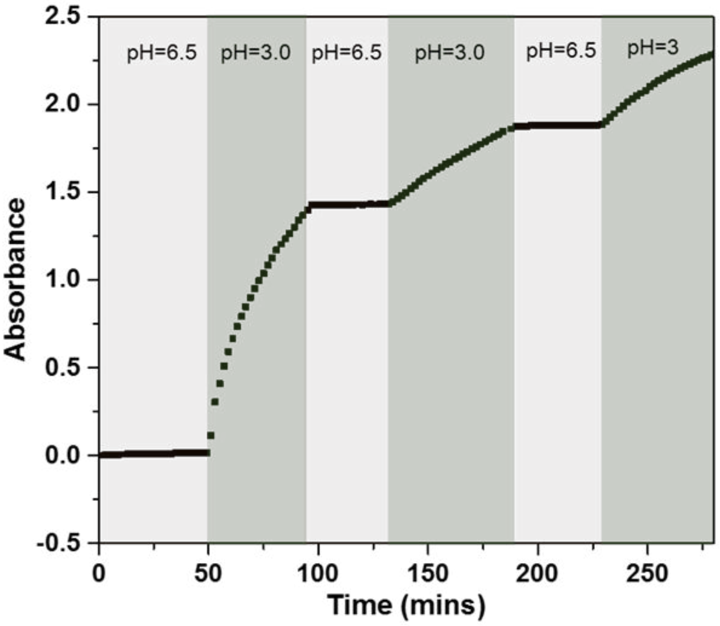

Figure 9. Controlled release of CV from the bilayers by switching solution pH.

3. Conclusion

In summary, pNIPAm-pDADMAC semi-IPN hydrogels/PDMS bilayers were prepared and we demonstrated that they are capable of bidirectional bending in response to solution temperature and pH. By careful investigation of the pore size of the hydrogel layers at the various conditions we were able to hypothesize that the behavior was a result of the swelling and shrinking of the hydrogel layer, while the PDMS layer remains unswellable. The bilayers exhibit reversible and repeatable thermoresponsive and pH-responsive bending/unbending characteristics. The bilayers were shown to act as soft grippers and were able to load and release small molecules in response to pH while still exhibiting a bending response. The behavior of these materials, combined with their soft mechanical properties could make them useful for various biomedical applications.

4. Experimental

Materials: N-isopropylacrylamide (NIPAm) was purchased from TCI (Portland, Oregon) and purified by recrystallization from hexanes (ACS reagent grade, EMD, Gibbstown, NJ) prior to use. N, N'-methylenebisacrylamide (BIS) (99%), acrylic acid (AAc) (99%), 2-propene-1-thiol (60% GC) and 2,2-Diethoxyacetophenone (DEAP) were obtained from Sigma-Aldrich (Oakville, Ontario) and were used as received. Poly (diallyldimethylammonium chloride) solution (pDADMAC) with molecular weights of 100,000 ~ 200,000 (low Mw), 200,000 ~ 350,000 (medium Mw), and 400,000-500,000 (high Mw) (20wt % in water) were purchased from Sigma-Aldrich (St. Louis, MO). Sylgard 184 silicone elastomer base and Sylgard 184 silicone elastomer curing agent were purchased from Dow Corning Corporation, Midland, MI, USA. Deionized (DI) water with a resistivity of 18.2 MΩ•cm was used. Cr/Au annealing was done in a Thermolyne muffle furnace from Thermo Fisher Scientific

(Ottawa, Ontario). Anhydrous ethanol was obtained from Commercial Alcohols (Brampton, Ontario). Cr was 99.999% and obtained from ESPI (Ashland, OR), while Au was 99.99% and obtained from MRCS Canada (Edmonton, AB).

Preparation of pre-gel solution: Three kinds of solutions were prepared for the fabrication of thermoresponsive bi-directional self-bending bilayers. In solution 1, we firstly mixed 12 mL DI water and 3 mL pDADMAC solution (20 wt%, low Mw), which was used as a "solvent". Then monomers mixtures were dissolved in this solvent, with a total monomer concentration of 7.89 mol/L. The monomer mixtures contain 95% (mol%) of NIPAm, and 5% BIS. Then, 40 μL DEAP (as a photoinitiator) was added to the solution, followed by covering the container of the solution with aluminum foil and shaking the solution for 1 h. Likewise, in solution 2 and 3, we used pDADMAC solution (20 wt%) with medium Mw and high Mw, respectively. For each solution, we made a control solution with no pDADMAC added.

Additionally, we made three solutions for the fabrication of pH responsive bi-directional self-bending bilayers. In solution 1, we firstly mix 12 mL DI water and 3 mL pDADMAC solution (20 wt%, low Mw), which is used as a solvent. Then monomer mixtures were dissolved in the solvent, with a total monomer concentration of 7.89 mol/L. The monomer mixtures contain 80% (mol%) of NIPAm, 15% AAc, and 5% BIS. Then 40 μL DEAP (as a photoinitiator) was added in the solution, followed by covering the container of the solution by aluminum foil and shaking the solution for 1 h. Likewise, in solution 2 and 3, we used pDADMAC solution (20 wt%) with medium Mw and high Mw, respectively. For each solution, we made a control solution with no pDADMAC added.

Fabrication of self-bending IPN hydrogel based bilayers: A PDMS film with a thickness of 0.5 mm was generated by mixing silicone elastomer base and curing agent (from Dow Corning) in a volume ratio of 10:1. Then the resulting film was rinsed with DI water and ethanol and dried with N_2 gas, and 2 nm of Cr followed by 50 nm of Au were thermally evaporated onto the PDMS at a rate of ~0.2 Å s-1 and ~0.1 Å s-1, respectively, using a Torr International Inc. model THEUPG thermal evaporation system. Then the Au-coated PDMS layer was soaked in an ethanolic solution of 1-propene-2-thiol overnight at 4 °C, followed by soaking in ethanol for more than 5 h to rinse away the excess 1-propene-2-thiol. Then cross-shaped substrates were cut out of the modified PDMS sheet. The distance between two neighbouring vertexes was 1.25 cm. Then the cross-shaped substrates were put on the surface of a Petri dish. After deposition of the pre-gel solution on the substrate, the Petri dish was placed onto a cooling plate (Stir-Kool Model SK-12, Thermoelectrics Unlimited, Inc.), which was supported by recycling cool water through the cooling plate. The temperature of the cooling plate was set to ~ 10 °C. Afterwards, the pre-gel solution was covered by a thin Teflon sheet. Then after UV irradiation for 15 min, photo-initiated polymerization led to the formation of hydrogels and IPN hydrogels. The formed hydrogel-based bilayers were released, followed by washing away the unreacted monomers by adding and changing DI water in the Petri dish for several days.

Field emission scanning electron microscopy: The bent bilayer systems were first frozen by immersion in liquid nitrogen for 10 min. Then the bilayers were freeze-dried overnight. (FreeZone. 4.5, LABCONCO). The dried bilayers were then placed on a conductive copper tape coated holder and SEM images acquired on specific areas using a Zeiss Sigma FESEM, operated at 5 kV.

Crystal violet (CV) loading and release from bilayers: Firstly, a bilayer film was generated by mixing 12 mL of DI water and 3 mL of pDADMAC solution (20 wt%, low Mw), which was used as a solvent. Then monomer mixtures were dissolved in the solvent, with a total monomer concentration of 7.89 mol/L. The monomer mixtures contain 80% (mol%) of NIPAm, 25% AAc, and 5% BIS. Then 40 μL DEAP (as a photoinitiator) was added in the solution, followed by covering the container of the solution by aluminum foil and shaking the solution for 1 h. The control solution contained the same components as above except no pDADMAC was present. The fabricated bilayers, which were generated using the same procedure as above, were firstly soaked in a solution of pH 3, which causes the bilayers to be almost flat. Then we placed the bilayer in a bottle containing 15 mL of CV solution (1 mg/mL, pH 6.5) and a polymeric target particle at the bottom. After overnight soaking, the bilayer was completely bent up towards the side of PDMS, and at the same time, the polymeric particle was encapsulated by the bilayer. For CV release, a glass vial containing 20 mL pH 6.5 solution was placed on a plate with the temperature set as ~ 25 °C. The solution was stirred continuously at 60 rpm using a magnetic stir bar and flowed through a cuvette in an Agilent 8453 UV-vis spectrophotometer, equipped with an

89090A temperature controller and Peltier heating device, via a peristaltic pump. The pumping speed was kept constant for the whole experiment. Then the CV loaded bilayer with the encapsulated polymeric particle was placed into the solution, and a timer started. After 30 mins, the pH of the solution was changed to 3. The absorbance spectrum from the solution was collected every 2 mins.

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