

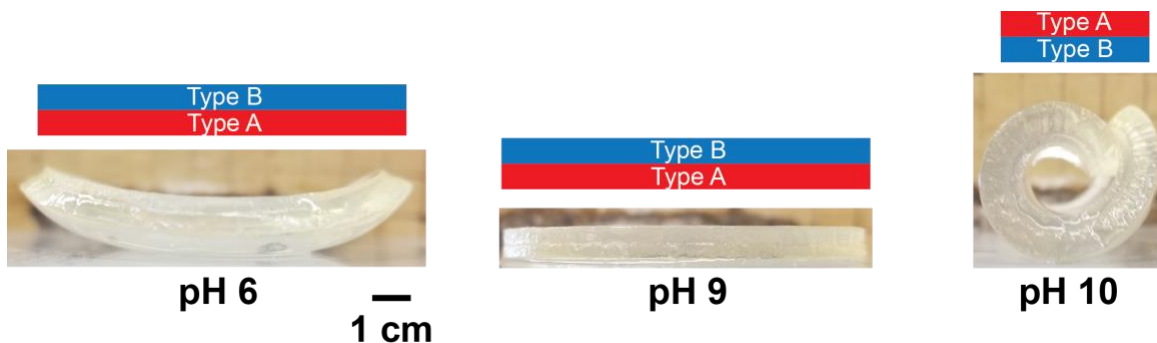
Mechanically cycling gelatin bilayers

Laura E. Hanzly¹, Natasha Chauhan², and Justin R. Barone^{1,3,4*}

¹Biological Systems Engineering, ²Chemical Engineering, ³Macromolecules Innovation Institute, ⁴Center for Soft Matter and Biological Physics
Virginia Tech
Blacksburg, VA 24061

ABSTRACT

There is a growing interest in making stimuli-responsive polymer systems, particularly ones that are bio-inspired/biomimetic and could perform mechanical work. Here, a biological device made from gelatin is described that can mechanically cycle back and forth in response to solution pH or ionic strength changes. The gelatin bilayer has one layer of Type A gelatin and the other of Type B gelatin, which have 2 different isoelectric points and therefore ionization states at a given solution pH. The bilayer mechanically cycles back and forth when one layer swells more than the other layer, which occurs because of solution pH or ionic strength change. Maximum bilayer bending occurs at pH 10, when the Type B gelatin layer swells significantly more than the Type A layer. The results show the ability to use the unique properties of different sources of gelatin to design a simple biological machine.



INTRODUCTION

Polymer bilayers that have homogenous surface area but are heterogeneous through the thickness can be designed to bend, curl, and twist in response to a stimulus (Figure 1).¹ If the length (l) is much longer than the width (w) and thickness (h) then the structure is considered “quasi-1D” (Figure 1a) while if the length is similar to the width and both are much longer than the thickness the structure is “quasi-2D” (Figure 1b).² The polymeric bilayers work in the same manner as bimetallic strips where one metal has a higher coefficient of thermal expansion than the other.³ In this case, a polymer gel bilayer is considered where one layer swells more than the other layer with bending towards the less swollen layer. The typical convention is to call the higher swelling layer the “active” layer and the lower swelling layer the “passive” layer.⁴ However, in some incarnations the active layer is simply a layer that swells or shrinks upon stimulation while the passive layer does nothing. The polymer gels are typically crosslinked or of very high molecular weight so they are highly entangled. This allows them to be solvent swollen without complete dissolution.

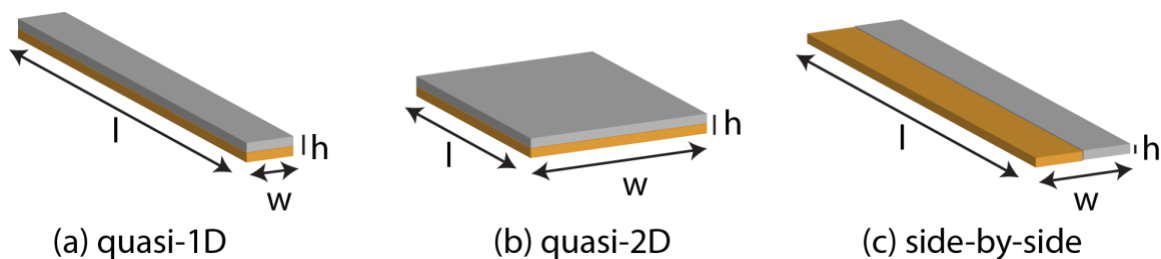


Figure 1. A polymer gel bilayer where each layer can be swollen to a different extent (orange and gray) can be formed into (a) quasi-1D with $l \gg w \sim h$ and (b) quasi-2D with $l \sim w \gg h$ geometries. (c) The layers can also be placed side-by-side in any configuration.

Bilayers of high swelling and low swelling gels can be made from a number of polymers.⁵ For example, bilayers of polyacrylamide (PAM) and poly(N-isopropyl acrylamide, PNIPAm)-PAM interpenetrating networks (IPNs) could be bent in both

directions with different stimuli.⁶ At temperatures above room temperature the IPN layer shrinks so bending will be towards that layer. In acetone-water solution, the PAM layer shrinks, with bending towards that layer. Temperature is sometimes used as a stimulus because in some cases heat transfer can be faster than mass transfer so the bilayer can bend and curl when the active layer swells at reduced temperature or shrinks at higher temperature.^{2,7} Bilayers of hydrophilic and hydrophobic polymers can bend in different directions when stimulated with temperature or solvent, depending on which layer is swollen more.⁸⁻¹¹ Charged polymers can also be used in the bilayer to get reversible actuation from changes in solution pH and ionic strength (IS).¹² Polymethacrylic acid (PMAA)-polyethylene glycol dimethacrylate (PEGDMA) bilayers can swell at pH values greater than the PMAA pK_a because the PMAA layer becomes highly negatively charged and the PEGDMA layer is highly crosslinked and not able to swell.⁵ PMAA-poly(hydroxyethyl methacrylate) (PHEMA) bilayers also bend at $pH > PMAA\ pK_a$ values because the PHEMA layer remains unchanged.¹³

Heterogeneously structured polymer gel concepts can be extended to other geometries to form flat 2D material “origami” that can change shape to 3D. Changing the structure shown in Figure 1 to now have heterogeneity in the surface area but none in the thickness (put the 2 differently swelling polymer gel layers next to each other rather than on top of each other, Figure 1c) will allow for more complex buckling and curling.^{14,15} A similar design can create 2 “legs” when a cationic hydrogel is joined end-to-end to an anionic hydrogel.¹⁶ The acrylamide/sodium acrylate anionic leg and acrylamide/quaternized dimethylaminoethyl methacrylate cationic leg will move under an electric field. The gels can also be formed into annular structures with one gel surrounding the other so there is

again heterogeneity in surface area but not in thickness.^{14, 15, 17} More complex geometries like saddle structures can be realized upon swelling. Lithography can pattern low swelling areas into a larger area gel of higher swelling to realize complex shape change like spheres and saddles.¹⁸ “Ionoprinting” can spatially pattern ions into hydrogels to create a heterogeneous structure that will respond to an electrical stimulus to realize shape change.¹⁹

The bilayer concept has been shown to be one used by nature to allow plants to grow and proliferate.²⁰ Hydrated pine cones are closed but will fall off the tree, dehydrate, and open to disperse seeds.⁴ The pine cone scale is a bilayer with differing amounts and orientations of cellulose fibrils in each layer that restrict swelling through the thickness allowing the scale to bend in (hydrated) or out (dehydrated). Seed pods have 2 halves with cellulose fibrils oriented $\pm 45^\circ$ to the seed pod long axis and thus 90° to each other. When hydrated the seed pod is shut but upon falling off the tree and dehydrating, the 2 halves will dehydrate and twist off one another, thus opening up and dispersing seeds.²¹ Cucumber tendrils will curl around objects and grow towards light because one half of the cross-section is lignified and the other is not, again allowing differential swelling through the thickness.^{22, 23} Pine cones and seed pods serve as inspiration to construct bilayers with microfibrils in one or both layers of the hydrogel bilayer. The orientation and amount of fibers will restrict swelling allowing for tight curling of quasi-1D rectangular strips into helices.²⁴⁻²⁶

The bilayer concept has been extended to building devices. Poly(allylamine hydrochloride), PAH, and poly(acrylic acid), PAA, deposited onto a polytetrafluoroethylene (PTFE) substrate create a bilayer where the PAH-PAA layer

swells in water and shrinks when dry.²⁷ Branched poly(ethylenimine)/poly(acrylic acid) (BPEI/PAA) bilayers coupled to poly(diallyldimethylammonium chloride)/polystyrenesulfonate (PDAC/SPS) bilayers will bend in ethanol as the BPEI/PAA layer shrinks and the PDAC/SPS layer swells.²⁸ A “ratchet”-type device built from the PAH-PAA/PTFE bilayer and incorporated into a small vehicle can move when the relative humidity changes.²⁷ Bilayers of PNIPAm-polyvinyl alcohol (PVA) and poly(2-(dimethylamino) ethyl methacrylate-poly(sodium-p-styrenesulfonate) (PDMAEMA-PSS) can be formed into reversible 4 armed grippers.²⁹ In acid, the PDMAEMA layer has high swelling from protonation of the amino group, so the bilayer bends towards the PNIPAm layer, which shrinks when the temperature is above its lower critical solution temperature (LCST). Reduction of the temperature below the PNIPAm LCST causes it to swell more and the hydrogel to bend in the other direction. Quasi-2D bilayer shapes such as stars, crosses, and discs can be made into grippers that can open and close to grab or encapsulate objects for later release.^{10, 12, 30, 31} Commercially available sodium polyacrylate (NaPA) beads glued to paper in different patterns could achieve different configurations when swollen in water from the highly swollen NaPA hydrogel relative to the less swollen paper.³²

It would be advantageous to use economical, commercially available, and biocompatible/generally regarded as safe (GRAS) materials for food and drug applications of stimuli-responsive hydrogels.³³ Gelatin is one such material and hydrogel bilayers of different crosslink density can be created that bend based on the resulting swelling difference.^{34, 35} Since a hydrogel is mostly water, biochemical reactions can occur. Addition of starch to a gelatin bilayer active layer will increase its swelling. If the

active layer also contains amylase, the amylase then hydrolyzes starch to smaller molecular weight sugars, which diffuse out. This returns the bilayer to its original state and allows for a cyclic device that works on a sugar input and biochemical reaction, much like a real biological system.³⁵ Here, that idea is extended to show that using 2 different gelatin types can create a system capable of bending in both directions. Depending on the collagen processing conditions, gelatin can exhibit drastically different ionic properties.³⁶⁻³⁸ It is hypothesized that the pI difference between Type A and Type B gelatin would allow for the creation of a soft mechanically cycling bilayer that responds differently at different solution pH or ionic strength.

EXPERIMENTAL SECTION

Single Layer Gels. Type A (G2500) and Type B (G9382) gelatin were purchased from Sigma Aldrich (St. Louis, MO). Single layer gelatin gels were prepared by first dissolving 20 wt% of either Type A or Type B gelatin in deionized water at 40°C. Once dissolved, the gelatin solution was placed in a vacuum oven to remove air bubbles. 5 ml of gelatin solution was then poured into a circular 5.5 cm diameter silicone mold and allowed to dry for 10 minutes. Gelatin gels were cut and removed from silicone molds using a 4.4 cm diameter circular metal cutter. Therefore, the gels had the quasi-2D geometry common in the literature to mimic actual mechanical devices, i.e., grippers, microencapsulators, and biological organisms like jellyfish, etc.

pH-Dependent Swelling Studies. Single layer gels were swollen in pH 2, 4, 6, 8, 10, and 12 solutions. pH solutions were prepared by adding either 1M NaOH or 1M HCl to 500 ml of deionized water. Gels were weighed after swelling for 48 hrs and then placed on Teflon-coated aluminum sheets to dry under laminar flow in a fume hood. The dry

weight of the gels was recorded after 48 hrs of drying. Swelling studies were performed in triplicate and results reported as average equilibrium volume swelling, Q , \pm standard error. Swelling ratios for pH 2 and 12 were not reported because the gelatin network was severely hydrolyzed.

The equilibrium volume swelling ratio, Q , is defined as the ratio of the total swollen volume attainable (polymer + water solvent), V_T , to the dry polymer volume, V_p :

$$Q = \frac{V_T}{V_p} = \frac{1}{v_p} = \frac{m_w \rho_{gel}}{m_d \rho_T} \quad (1)$$

where v_p is the polymer volume fraction in the water swollen hydrogel.³⁹ Q is found from the solvent swollen gel mass, m_w , the dry gel mass, m_d , the density of the gelatin ($\rho_{gel}=1.34 \text{ g/cm}^3$)⁴⁰, and the total density of the water swollen gelatin, ρ_T . ρ_T can be calculated from:

$$\rho_T = \left[\frac{1}{\rho_{gel}} \frac{m_d}{m_w} + \frac{1}{\rho_w} \left(1 - \frac{m_d}{m_w} \right) \right]^{-1} \quad (2)$$

where $\rho_w=0.997 \text{ g/cm}^3$ is the density of water at room temperature.

Gel Bilayers. Bilayer gels were formed by depositing single layers of gelatin on top of one another. 5 ml of 20 wt% Type B gelatin solution (as previously described) was first poured into the silicone mold. After allowing this first layer to dry for 10 minutes, 5 ml of 20 wt% Type A gelatin solution was poured directly on top of the Type B gelatin layer in the silicone mold. Once dry, gelatin bilayers were cut and removed from the mold using the same 4.4 cm circular cutter used to prepare single layer gels.

Bilayer Swelling and Curvature Measurements. Gelatin bilayers were allowed to swell for 48 hours in fresh solutions of the same pH values used for the single layers. Special attention was given to ensure samples had enough space to swell/bend with no

obstruction. The active layer was always on the bottom and the passive layer was always on the top so the bending was upwards to avoid any issues with friction or gravity opposing the bending.³⁵ After 48 hours, bilayers were immediately photographed upon removal from the water. On swelling the quasi-2D geometry, each bilayer had two principal curvatures: the maximum of the normal curvature, κ_1 , and the minimum of the normal curvature, κ_2 . Fiji⁴¹ software was used to measure the radius of curvature at the bilayer midplane for each principal curvature with $\kappa_1=1/r_1$ and $\kappa_2=1/r_2$ (Figure 2a).^{2, 17, 35} At low curvature, the bilayers displayed $\kappa_1=\kappa_2$ but then bifurcated with $\kappa_1 \gg \kappa_2 \sim 0$, thus behaving more as quasi-1D systems (Figure 2b).² Therefore, an average maximum principal curvature, κ_1 , was calculated from 3 replicates of each bilayer formulation and was reported along with the standard error. Single layer controls were also swollen and checked for bending but none was observed.

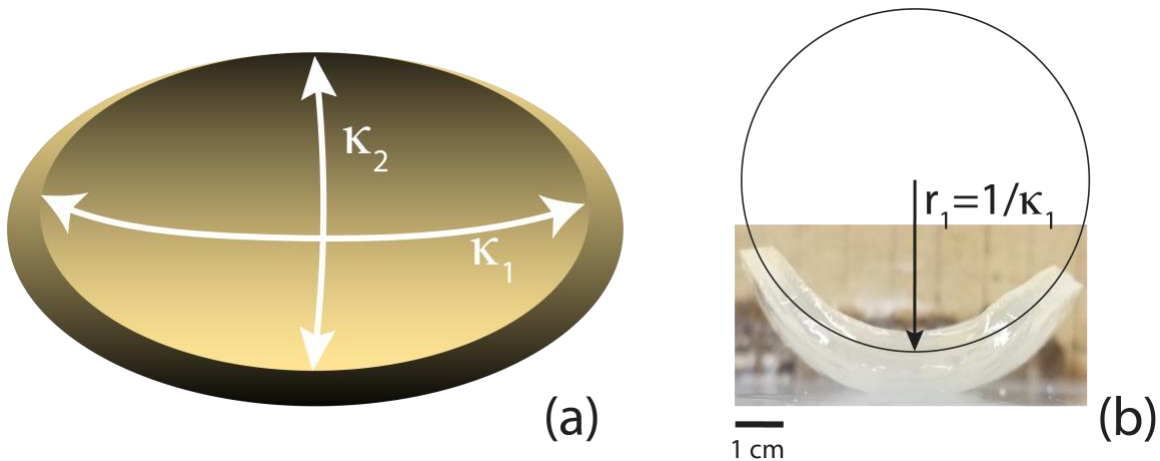


Figure 2. (a) A quasi-2D disk has two principal curvatures: the maximum of the normal curvature, κ_1 , and the minimum of the normal curvature, κ_2 . (b) The disk (side view) maximum principal curvature, κ_1 , is reported where $\kappa_1=1/r_1$. The maximum radius of curvature, r_1 , is defined as the radius of the circle that can be drawn through the curved bilayer midplane along the axis of largest curvature (κ_1).

Cyclic Actuation Experiments. Gelatin bilayers were first swollen in pH 6 solution for 48 hrs. The bilayers were then placed in pH 10 solution for another 48 hrs. NaCl was then directly added and dissolved into the pH 10 solution beakers. Bilayers were incubated in the salt solutions for 24 hrs after which they were placed in fresh pH 10 solution for another 48 hrs. The times were artificially long to ensure an equilibrium swelling was achieved. At each pH change and salt addition, the bilayers were removed from the solution and photographed to measure the curvatures. Experiments were performed in triplicate and average maximum principal curvature, κ_1 , \pm standard error reported for each salt concentration.

RESULTS AND DISCUSSION

Individual Layer pH Swelling Studies. Acid hydrolysis is acceptable for less crosslinked collagens like pigskin and young collagen (10-48 hour hydrolysis) while alkaline hydrolysis is needed for more crosslinked bovine collagen (several weeks hydrolysis).⁴² Acid-hydrolyzed Type A collagen has a reported isoelectric point, pI, very similar to native collagen, pI=7.0-9.5. However, alkaline-hydrolyzed Type B collagen converts asparagine and glutamine amino acids to aspartic and glutamic acid, respectively, lowering the pI to 4.7-5.3.^{37, 42-44} Type A and B gelatin hydrogels show solution pH-dependent swelling behavior (Figure 3). The swelling should be the lowest around the pI because of gelatin's net neutral charge. The further away from the pI, the higher the swelling because of like charge repulsion along the gelatin molecules (with net positive charge below the pI and net negative charge above the pI). Types A and B gelatin both attain a similar volume swelling ratio of $Q \sim 15$, their lowest equilibrium volume swelling ratio, at pH of 10 and 6, respectively, suggesting the actual pI is slightly

higher than reported. Thus, at all pH except around pH=8-9, the two gelatins have different equilibrium volume swelling ratios.

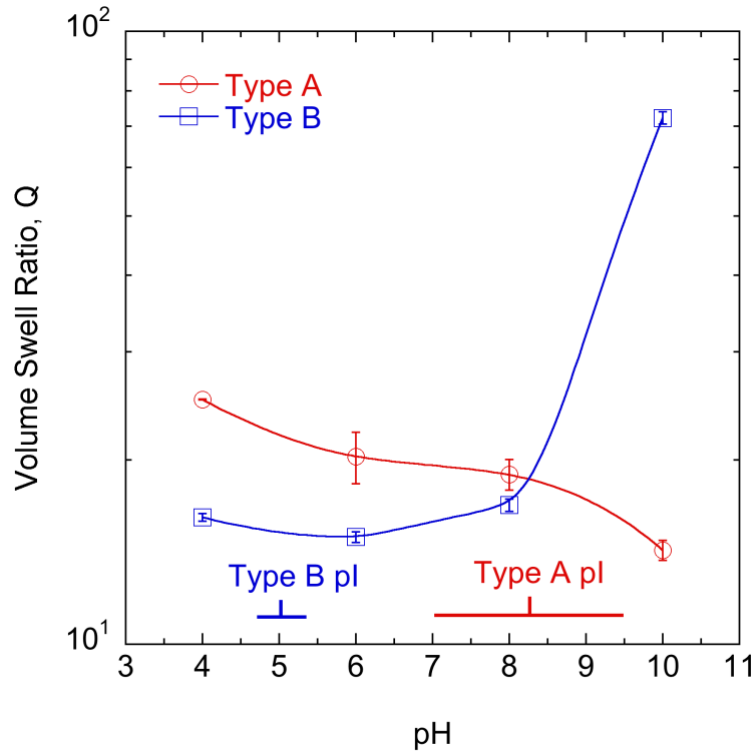


Figure 3. Average equilibrium volume swelling ratio, Q , of Type A and Type B gelatin layers at various pH. The reported pI is also shown.

Bilayer pH Swelling Studies. Bilayer actuating gels are fabricated with one layer of Type A and the other of Type B gelatin. The “active” and “passive” layers in the bilayer are the higher and lower swelling layers, respectively.⁴ Bending is always towards the less swollen passive layer (Figure 4a,b). At pH=4, 6, and 8, the Type A active layer with volume swelling ratio Q_a swells more than the Type B passive layer with volume swelling ratio Q_p . At pH=10, the Type B active layer swells more than the Type A passive layer so Q_a and Q_p are switched compared to the other pH values. Bilayer curvature depends on how swollen the passive layer is relative to the active layer, Q_p/Q_a .³⁵ When $Q_p/Q_a \rightarrow 1$ there is not enough volume swelling difference between the

layers for the bilayer to bend, which is what happens at pH=8 and 9, resulting in low to no curvature.³⁵ The data fit in Figure 3 suggests there should be a volume swelling difference at pH=9 but the low bilayer curvature data shows that there still is not a large volume swelling difference until pH>9 indicating that Q crosses over between Type A and B gelatin closer to pH=90. At $0 < Q_p/Q_a < 1$, the more swollen active layer is able to bend the less swollen passive layer resulting in high bilayer curvature. Since Q_a and Q_p switch depending on the solution pH, the bilayer could potentially bend back and forth when stimulated with a pH change (Figure 4a,b). Eventually, as $Q_p/Q_a \rightarrow 0$, the highly swollen and very soft active layer is not able to bend the much less swollen and much stiffer passive layer and there will again be no to low curvature.

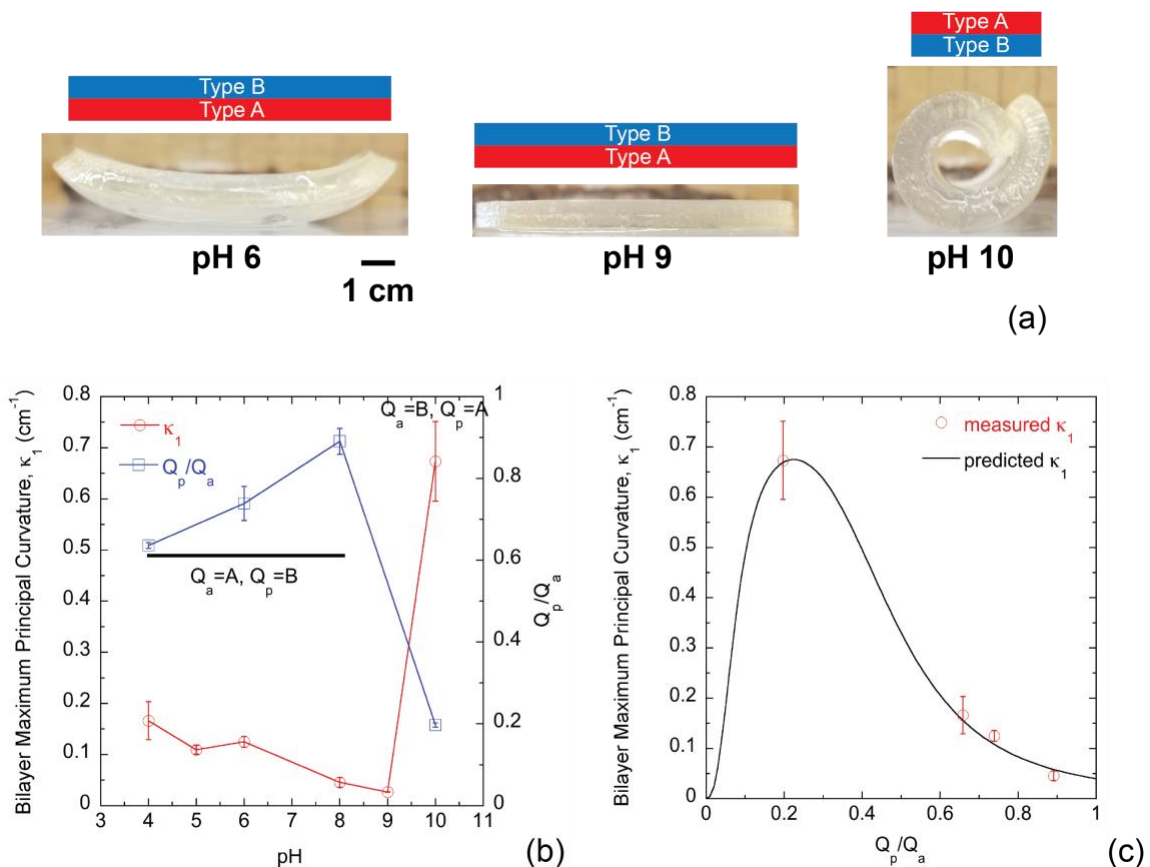


Figure 4. Curvature of bilayers as a function of solution pH. (a) Pictures of gelatin bilayers swollen for 48 hours at pH 6, 9, and 10. Note that the bilayer at pH=10 curls in

the direction opposite to the bilayers at pH=6, 9. (b) Average curvature for bilayers after 48 hours of swelling in different pH solutions. Q_p/Q_a is the volume swell ratio of the passive layer compared to the active layer. For pH values 4, 6, and 8, Type A gelatin is the active layer because it swells more than Type B gelatin. At pH 10, Type B gelatin is the active layer because it swells more than the Type A layer. (c) Hygromorph model fit to the curvature data.

Reyssat and Mahadevan developed a model to predict the curvature for bilayer hydrogels or “hygromorphs” based on the Timoshenko metallic bilayer model.^{3, 4} The model depends on the thickness ratio, $m=h_p/h_a$, and modulus ratio, $n=E_p/E_a$, of the two layers in the bilayer where E is elastic modulus. For $m=1$, curvature changes very little for a wide range of n because there is no swelling difference. When $n=0$ or ∞ , the active layer or passive layer, respectively, is too stiff to cause bending, which is similar to our observations for Q_p/Q_a here and in previous work.³⁵ For solvent swollen crosslinked elastomers, the m and n ratios are embodied by the ratio Q_p/Q_a . Elastic shear modulus, G' (hydrogels are soft so bulk modulus is much higher than shear modulus and Poisson's ratio=0.5 so $E=3G'$), and Q are inversely proportional, which can be found by inserting $G'=\rho_T RT/M_c$ (where R is the gas constant, T is absolute temperature, and M_c is the molecular weight along the polymer molecule between crosslinks) into the Flory-Rehner theory where for modest swell ratios (where the Gaussian polymer molecule assumption holds) it scales as $G' \sim Q^{-5/3}$.^{45, 46} Thus it is possible to use Q_p/Q_a in the hygromorph model to find curvature. Unfortunately, the theoretical $G' \sim Q^{-5/3}$ scaling does not produce adequate results. This is because the hydrogels are highly swollen and the scaling breaks down because of the loss of the Gaussian assumption in the theory. The actual scaling for highly swollen gelatin hydrogels is much higher, which is empirically observed and does not contain the same fundamental meaning as Flory's scaling (Figure S1). Using

$n=G'_p/G'_a=0.007(Q_p/Q_a)^{-5.5}$ and $m=(Q_p/Q_a)$ provides a good fit to the experimental data (Figure 4c and Supplemental Information, SI).

Cyclic Bilayer Actuation. A major drawback of some soft actuators is irreversible shape change.⁴⁷ This bilayer can bend in one direction when Type A gelatin is the active layer and the other direction when Type B gelatin is the active layer, which depends on solution pH (Figure 4). Therefore, mechanical cycling, i.e., back and forth bilayer motion, is possible if the solution pH changes with time. This is similar to a polyacid and polybase bilayer constructed from synthetic triblock copolymers.⁴⁸ The results in Figure 4 are obtained on new bilayers swollen in fresh solution of a single pH (i.e., pH is not changed with time). It is found that bilayer actuation cannot be reversed by placing the gelatin bilayer in a solution of one pH and then another (Figure 5). For instance, the curvature displayed by a gelatin bilayer at pH=10 cannot be reversed by placing the bilayer in a pH=6 or a pH=4 solution. The same thing happens when going from pH=6 to pH=10 solution. First, the much higher swelling of the active layer strains the much less swollen passive layer too much and breaks it resulting in permanent deformation of the passive layer, which leaves the bilayer permanently bent.³⁵ Plastic deformation in the passive layer also results in permanent bending in thermoplastic elastomer bilayers.⁴⁹ Second, at low and high pH gelatin's collagen fragments further hydrolyze resulting in lower molecular weight, lower triple helix content (crosslinks), and lower modulus.⁵⁰ This actually degrades both layers in the bilayer.

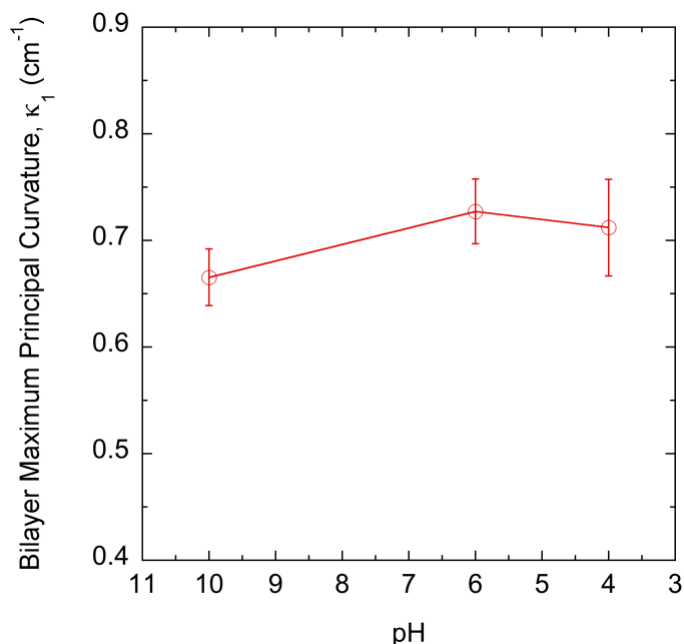


Figure 5. Bilayer curvature does not change appreciably when bilayers are moved from pH 10 to pH 6 solution and then from pH 6 to pH 4 solution.

To demonstrate mechanical cycling and also characterize the extent of network degradation in each layer throughout the cycle, bent bilayers are transitioned through pH then through fresh and NaCl-containing solutions at constant pH (Figure 6). At pH=6 the bilayers are bent towards the less swollen Type B passive layer (note that κ_1 is slightly lower here than for the single pH results described in Figure 4). The curvature is not very high at pH=6 because Q_p/Q_a is relatively high. At pH=10, the bilayer bends in the other direction when the Type B layer becomes the active layer because it swells much more than the Type A layer. Q_p/Q_a is lower at pH=10 resulting in a much higher curvature than at pH=6. At constant pH=10, curvature then decreases after placing the bilayers in solutions of NaCl of various ionic strength, which completes 1 mechanical cycle. The NaCl screens the charge on both layers but the charge screening is more pronounced for the highly swollen Type B active layer and Q_p/Q_a increases dramatically (the two layers have similar low equilibrium volume swelling ratios, Table S1). The cycle can be started

again by taking the bilayers out of salt solutions and placing them back into fresh pH=10 solutions to remove the NaCl, re-ionize the gelatin, and create a large swelling difference, Q_p/Q_a , again. 0.05M and 0.10M NaCl appear to fully screen the charge in the active Type B gelatin layer because the curvature is very low and Q_p/Q_a is high. 0.01M NaCl does not fully screen the charge because the curvature does not decrease to the same extent as the higher NaCl concentrations.

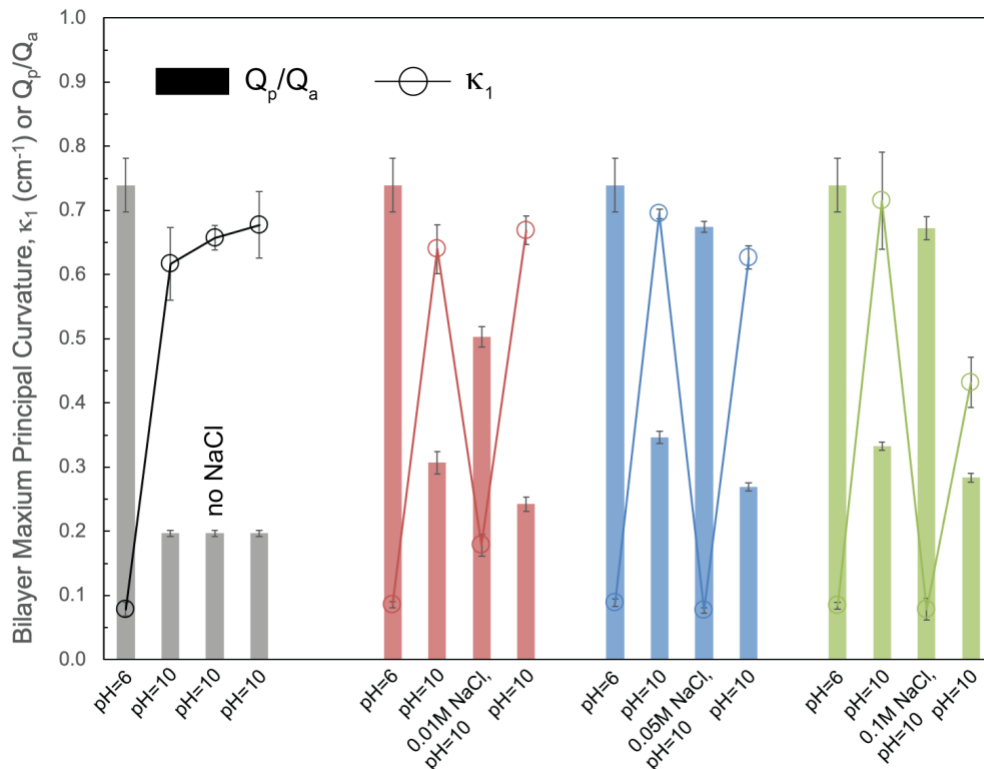


Figure 6. Gelatin bilayer mechanical cycling. Gelatin bilayers are first swollen at pH=6 where they bend towards the less swollen Type B gelatin passive layer and have low curvature. Bilayers are then placed at pH=10 where they bend towards the less swollen Type A passive layer and they show a much higher curvature. Bilayers are then placed in either 0.01M, 0.05M, or 0.10M NaCl solutions where each layer contracts from charge screening and the bilayer straightens. Finally, bilayers are placed back into pH=10 solutions to re-bend. The control group is kept in a constant solution of pH=10 without addition of NaCl (black symbols).

Gelatin network degradation is apparent from the individual layer equilibrium volume swelling ratio data, Q , measured during the mechanical cycling experiment (Table S1). Q

is lower for both gelatin types when first placed into pH=10 solution than when re-placed into pH=10 solution after the NaCl solution. The Q increase at pH=10 is much more pronounced for the Type B active layer than the Type A passive layer. Since Q increases for the same sample throughout the cycle, there is clearly network breakage from remaining too long at an elevated pH and/or plastically deforming the sample during mechanical cycling. Interestingly, it appears to be the latter because after 1 week at pH=10, κ_1 has not changed much and similar results have been observed for gelatin bilayers that are highly strained but not exposed to high or low pH (Table S2).³⁵ If there is significant layer degradation over time, especially in Type B Q_p , κ_1 would change a lot.

CONCLUSIONS

A gelatin bilayer system capable of mechanical cycling was created by utilizing the isoelectric point difference of acid-hydrolyzed Type A gelatin and base-hydrolyzed Type B gelatin. Type A and Type B gelatin layers swelled to different capacities at different pH values, so that when constructed into a bilayer the bilayer would bend to different extents. The bilayer system had a maximum curvature at pH=10, when the volume swelling difference between the two layers was the highest. Mechanical cycling was achieved by switching the bilayers between different pH and ionic strength solutions. For instance: 1) At pH=6 the bilayer bent towards the less swollen Type B gelatin passive side, 2) at pH=10 the bilayer bent towards the less swollen Type A gelatin passive side, 3) upon the addition of NaCl at pH=10 the Type B gelatin active layer contracted more than the Type A gelatin passive layer from charge screening and the equilibrium volume swelling ratio was close to that of the Type A passive layer ($Q_p/Q_a \rightarrow 1$) resulting in curvature decrease, and finally 4) when placed in fresh pH=10 solution the bilayer re-bent

to a high curvature when it regained the repulsion along the gelatin chain and the Type B gelatin active layer increased its volume much more than the Type A passive layer. Network breakage was observed throughout the cycle during swelling and bending.

ASSOCIATED CONTENT

Supporting Information

Experimental shear modulus-equilibrium volume swelling ratio (G' - Q) relationship; Reyssat and Mahadevan hygromorph model parameters; equilibrium volume swelling ratio and curvature data for mechanically cycling bilayers.

AUTHOR INFORMATION

Corresponding Author

Justin R. Barone - *Biological Systems Engineering, Macromolecules Innovation Institute, Center for Soft Matter and Biological Physics, Virginia Tech, Blacksburg, VA 24061*; ORCID: orcid.org/0000-0001-8869-2475; Phone: (540) 231-0680; Email: jbarone@vt.edu.

Authors

Laura E. Hanzly - *Biological Systems Engineering, Virginia Tech, Blacksburg, VA 24061*; *current address: Bioengineering, UMass-Dartmouth, Dartmouth, MA 02747*

Natasha Chauhan - *Chemical Engineering, Virginia Tech, Blacksburg, VA 24061*

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SUPPORTING INFORMATION

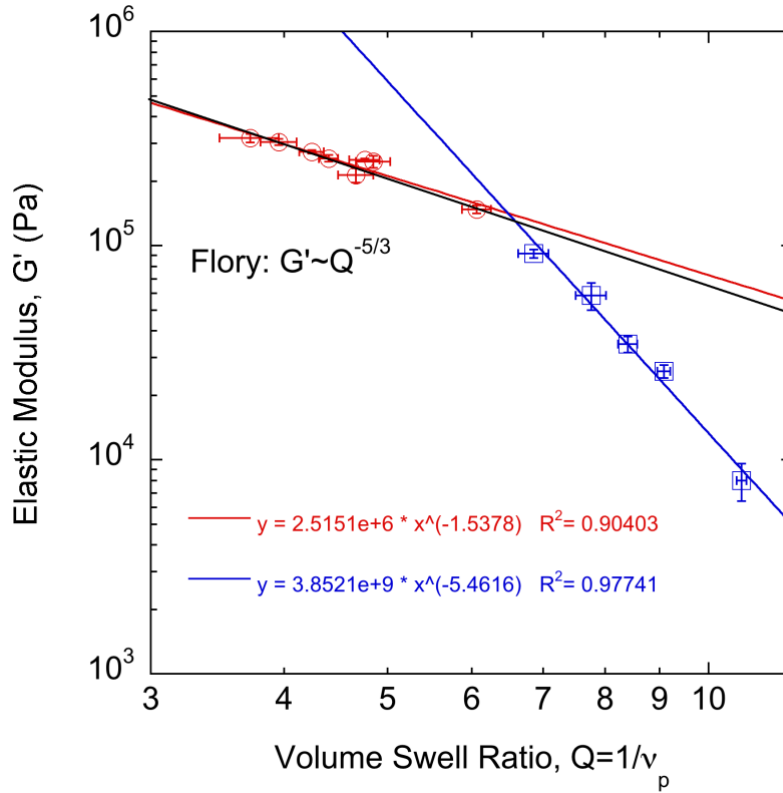


Figure S1. The elastic shear modulus scales with the equilibrium volume swelling ratio at moderate Q (up to about 6) according to the Flory-Rehner theory, $-5/3$ (red). At high equilibrium volume swell ratio, the scaling is much higher, -5.5 (blue).

In the Reyssat and Mahadevan hygromorph model, $m=h_p/h_a$, which is the thickness ratio of the two layers. For the cylindrical swollen hydrogel layer in this study, the thickness is $h=V_T/\pi r^2$, where $V_T=QV_p$ (Equation 1) and r is the cylinder radius. Therefore, $h=QV_p/\pi r^2$. The starting dry volume of Type A and B gelatin in each layer is the same, $V_{p,p}=V_{p,a}=\text{constant}$. When both layers are attached into a bilayer, πr^2 is constant. Upon swelling, it is the change in thickness between the two layers that drives the bending. If r changed in each layer too then the bilayer would not bend because the radius would also accommodate the swelling and the bilayer would just expand in three dimensions. Therefore, $h_p=Q_p V_{p,p}/\pi r^2$ and $h_a=Q_a V_{p,a}/\pi r^2$. Thus, $m=h_p/h_a=Q_p/Q_a$. It is

empirically found that $G' \sim Q^{-5.5}$ in the very high swelling regime for gelatin (Figure S1). Therefore, $n = G'_p / G'_a = (Q_p / Q_a)^{-5.5}$. The 0.007 prefactor in $n = 0.007(Q_p / Q_a)^{-5.5}$ provides the best fit with the prefactor originating in the empirical $G' \sim Q$ relationship at very high Q . The m and n values based on Q_p / Q_a are used in Reysat and Mahadevan's $f(m, n)$ function in the model. The curvature change is $\Delta\kappa = (\alpha\Delta\phi/h) * f(m, n)$, where $h = h_p + h_a$, $\Delta\phi$ is the change in hydration, and $\alpha = \alpha_a - \alpha_p \neq 0$ is the linear hygrometric expansion coefficient difference such that the different swelling of both layers results in bending. Here, $(\alpha\Delta\phi/h) = 0.5$ gives the best fit and defines the strain in the bilayer.

Table S1. Equilibrium volume swelling ratio changes for mechanically cycling bilayers in Figure 6.

Sample	Solution	Q (Type B)*	Q (Type A)*
Control	pH=6	14.96 ± 0.30	20.24 ± 1.94
	pH=10	72.24 ± 1.64	14.24 ± 0.53
	pH=10, no NaCl	72.24 ± 1.64	14.24 ± 0.53
	pH=10	72.24 ± 1.64	14.24 ± 0.53
0.01M NaCl	pH=6	14.96 ± 0.30	20.24 ± 1.94
	pH=10	36.97 ± 1.27	11.35 ± 0.51
	pH=10, 0.01M NaCl	20.54 ± 0.37	10.33 ± 0.28
	pH=10	57.63 ± 1.72	13.96 ± 0.48
0.05M NaCl	pH=6	14.96 ± 0.30	20.24 ± 1.94
	pH=10	31.78 ± 0.68	11.02 ± 0.19
	pH=10, 0.05M NaCl	16.81 ± 0.17	11.34 ± 0.08
	pH=10	47.91 ± 0.93	12.92 ± 0.17
0.1M NaCl	pH=6	14.96 ± 0.30	20.24 ± 1.94
	pH=10	32.21 ± 0.61	10.71 ± 0.04
	pH=10, 0.1M NaCl	17.53 ± 0.46	11.78 ± 0.01
	pH=10	53.22 ± 1.24	15.10 ± 0.07

*At pH=6, Type B gelatin is Q_p and Type A gelatin is Q_a . At pH=10, Type B gelatin is Q_a and Type A gelatin is Q_p .

Table S2. Bilayer curvature, κ_1 , data through mechanical cycling.

Solution	No NaCl	0.01M NaCl	0.05M NaCl	0.1M NaCl
pH=6	0.079 ± 0.010	0.086 ± 0.005	0.089 ± 0.006	0.084 ± 0.005
pH=10	0.617 ± 0.056	0.640 ± 0.038	0.695 ± 0.008	0.715 ± 0.076
pH=10+NaCl	0.657 ± 0.019	0.178 ± 0.017	0.077 ± 0.004	0.079 ± 0.017
pH=10	0.677 ± 0.052	0.669 ± 0.022	0.627 ± 0.018	0.432 ± 0.039
pH=10, 1 week	0.728 ± 0.054	0.749 ± 0.025	0.712 ± 0.023	0.431 ± 0.033

Data used to construct Figure 6; 1 week data omitted in the figure for clarity.