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Enzyme-Triggered Folding of Hydrogels: Toward a Mimic of the Venus Flytrap

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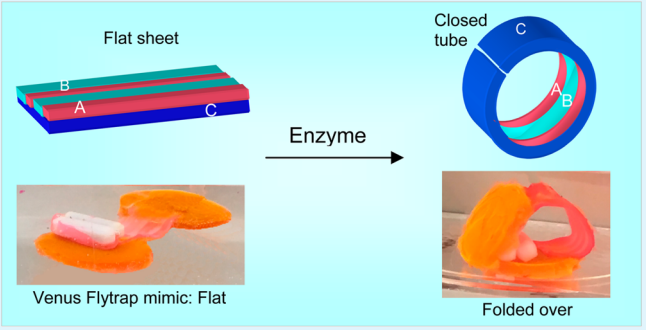
Supporting Information

ABSTRACT: External triggers such as pH or temperature can induce hydrogels to swell or shrink rapidly. Recently, these triggers have also been used to alter the three-dimensional (3-D) shapes of gels: for example, a flat gel sheet can be induced to fold into a tube. Self-folding gels are reminiscent of natural structures such as the Venus flytrap, which folds its leaves to entrap its prey. They are also of interest for applications in sensing or microrobotics. However, to advance the utility of self-folding gels, the range of triggers needs to be expanded beyond the conventional ones. Toward this end, we have designed a class of gels that change shape in response to very low concentrations of specific biomolecules. The gels are hybrids of three different constituents: (A) polyethylene glycol diacrylate (PEGDA); (B) gelatin methacrylate-*co*-polyethylene glycol dimethacrylate (GelMA-*co*-PEGDMA); and (C) *N*-isopropylacrylamide (NIPA). The thin-film hybrid is constructed as a bilayer or sandwich of two layers, with an A/B layer (alternating strips of A and B) sandwiched above a layer of gel C. Initially, when this hybrid gel is placed in water, the C layer is much more swollen than the A/B layer. Despite the swelling mismatch, the sheet remains flat because the A/B layer is very stiff. When collagenase enzyme is added to the water, it cleaves the gelatin chains in B, thus reducing the stiffness of the A/B layer. As a result, the swollen C layer is able to fold over the A/B layer, causing the sheet to transform into a specific shape. The typical transition is from flat sheet to closed hollow tube, and the time scale for this transition decreases with increasing enzyme concentration. Shape transitions are induced by enzyme levels as low as 0.75 U/mL. Interestingly, a shape transition is also induced by adding the lysate of murine fibroblast cells, which contains enzymes from the matrix metalloproteinase (MMP) family at levels around 0.1 U/mL (MMPs are similar to collagenase in their ability to cleave gelatin). We further show that transitions from flat sheets to other shapes such as helices and pancakes can be engineered by altering the design pattern of the gel. Additionally, we have made a rudimentary analog of the Venus flytrap, with two flat gels ("leaves") flanking a central folding gel ("hinge"). When enzyme is added, the hinge bends and brings the leaves together, trapping objects in the middle.



KEYWORDS: *responsive hydrogels, hybrid hydrogels, shape transformation, biomimetic materials, enzymatic degradation, cell lysate*

INTRODUCTION

Polymer hydrogels, made by the polymerization of monomers and cross-linkers, constitute a widely studied class of soft materials.[1−4] Such gels are three-dimensional networks of polymer chains connected at junction points by covalent bonds (cross-links). When placed in water, these gels swell to a volume that is much larger than their dry volume.[5] A striking feature of many gels is that their volume sharply changes in response to external stimuli such as temperature or pH.[3,4] Such volume changes can be discontinuous (step-like) and reversible. For example, gels of *N*-isopropylacrylamide (NIPA) remain swollen at low temperatures up to 32 °C, but shrink at temperatures above 32 °C. Thus, NIPA gels show a discontinuous response to temperature at 32 °C, which is the lower critical solution temperature (LCST) of NIPA.[3,4] In addition to changes in gel volume, which occur isotropically in the entire material, gels can also be made to undergo specific changes in their shape.[6−9] For example, flat gel sheets have been induced to transform into open helices or closed helical tubes in response to temperature, pH, or light.[6−15]

One motivation to study hydrogels is the existence of many soft gel-like materials in nature.[7,8] These include creatures that live in water such as squids and jellyfish as well as land animals such as worms. Additionally, the natural inspiration for shape-changing gels comes from plants. The prototypical example of a plant that changes shape is the Venus fly trap,[16−21] an

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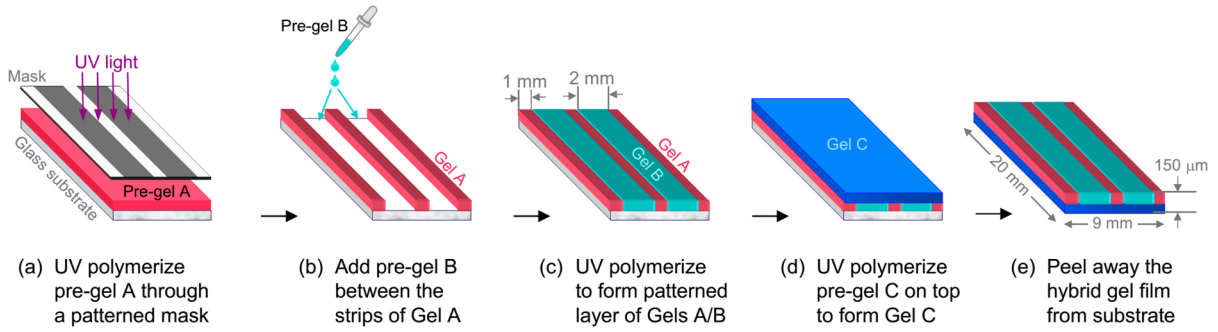

Figure 1. Synthesis of hybrid gels having three components by UV-photolithography. Gel A is PEGDA, Gel B is a copolymer of GelMA and PEGDMA, and Gel C is NIPA cross-linked with laponite nanoparticles. The various steps to form the hybrid gel are indicated in panels a to e in the figure. The final gel shown in panel e is a thin, rectangular film with the dimensions indicated, and it is a sandwich of two layers. The top layer has alternating strips of Gel A and Gel B, whereas the bottom layer is Gel C.

insectivorous plant with two large leaves that form a cup or wedge shape (see photos later in the paper). When an insect lands on the inner portion of the cup, the two leaves fold inward, trapping the insect, which is thereafter digested by the plant. Other examples of shape-changing plant-based materials include wheat awns and seedpods.[22,23] The driving force for shape changes in plants often comes from the differences in swelling ratio (i.e., turgor pressure) between adjacent types of plant tissue. Thus, it is important to recognize that soft materials in nature are not homogeneous; on the contrary, they can have many different zones with distinct chemical and mechanical properties. In creating biomimetic responsive gels, one should therefore look to mix and match different kinds of gels within a single hybrid material. Recently, several techniques have emerged to create such hybrid gels including soft lithography,[11] ionoprinting,[24] and a method introduced by our lab to create three-dimensional (3-D) hybrids by polymerization of viscous monomers.[25]

In this paper, we report the synthesis of hybrid gels that change shape in response to specific biomolecules (enzymes) at low concentrations. The shape changes we demonstrate include the folding of a flat gel sheet into a closed tube and the transition of a Venus flytrap analog from an open to a closed state. Although several studies have explored the use of biomolecules as triggers for gels,[26−28] most of these studies have been conducted in the context of molecularly imprinted gels, where the binding of biomolecules merely induces the gel to swell or shrink. To our knowledge, the only biomolecule-triggered shape change reported in small objects was in the work of Gracias et al.,[29] which was on a complex material made by electron-beam lithography that contained both metallic portions as well as a hinge made from a polymer gel.

Biomolecular binding or enzymatic reactions have many unique features: for one thing, they are highly specific, i.e., they occur in a lock−key fashion between enzyme−substrate or biomolecule−ligand.[30] Also, very low (micro- to nanomolar) concentrations of biomolecules are adequate to induce substantial responses at the cellular level.[28,30] Thus, the challenge in our study was not only to drive a large macroscopic change in gel shape using biomolecules but to do so using minuscule concentrations of these molecules in solution. To achieve this, we have designed a A/B/C hybrid gel, as described below, where A, B, and C correspond to distinct chemistries, and only one of the three undergoes an enzymatic reaction. The enzyme-induced change, in turn, creates differential stresses in the gel sheet, which ultimately causes the sheet to change shape.[31] We expect such shape-changing gels to be of interest to researchers working in a variety of areas, including in soft robotics, in the design of biomimetic materials, and in the creation of biosensing platforms and biomedical implants. As one example, we show that the shape-changing property of these gels could serve as a macroscopic signal for the presence of certain physiologically relevant enzymes.

RESULTS AND DISCUSSION

Fabrication of Hybrid Gel Sheets. The hybrid gels designed here contain three different cross-linked polymers, designated as A, B, and C in Figure 1. These polymers are confined to specific zones in the overall hybrid. The hybrid has two thin layers, each about 75 μm thick, that are sandwiched together to form a "bilayer". The bottom layer has gels A and B arranged in alternating strips. The top layer is composed only of gel C. Each of these polymers was chosen based on different considerations. Gel A is based on polyethylene glycol diacrylate (PEGDA), which is a monomer with two cross-linkable acrylate groups. Gels of PEGDA are relatively stiff and do not swell much. Gel B is a copolymer of gelatin methacrylate (GelMA) and polyethylene glycol dimethacrylate (PEGDMA). The GelMA is the one material in our study that is sensitive to biomolecules. It is obtained by attaching methacrylate groups to the backbone of gelatin using a method reported in the literature.[32,33] Figure S1 (SI) shows the reaction scheme as well as evidence from ^{1}H NMR for the modification. From the spectra, the degree of methacrylation in the GelMA used here is estimated to be about 60%. The GelMA is cross-linked together with PEGDMA (two methacrylate groups) to form Gel B. Finally, Gel C is based on *N*-isopropylacrylamide (NIPA). To cross-link NIPA, rather than use a bifunctional monomer like bis-acrylamide (BIS), we use nanoparticles of a synthetic clay called laponite (these are disks with a diameter of 25 nm and a thickness of 0.9 nm).[25,34] Laponite (LAP) disks serve as cross-linking junctions, i.e., the polymer chains extend from one face of a disk to another.[34] The reason for using laponite is that the resulting gels are more flexible than those formed with bifunctional monomers.[15,25]

We utilized UV-photolithography to fabricate our hybrid gel sheets (Figure 1). A homemade photomask consisting of alternating black (2 mm wide) and clear (1 mm wide) strips was printed on transparencies. Ultraviolet (UV) light passes through the clear areas whereas it cannot penetrate the black ones. First, we spread a pregel solution of Gel A (i.e., 25 wt % PEGDA and initiator) between two glass slides and exposed it

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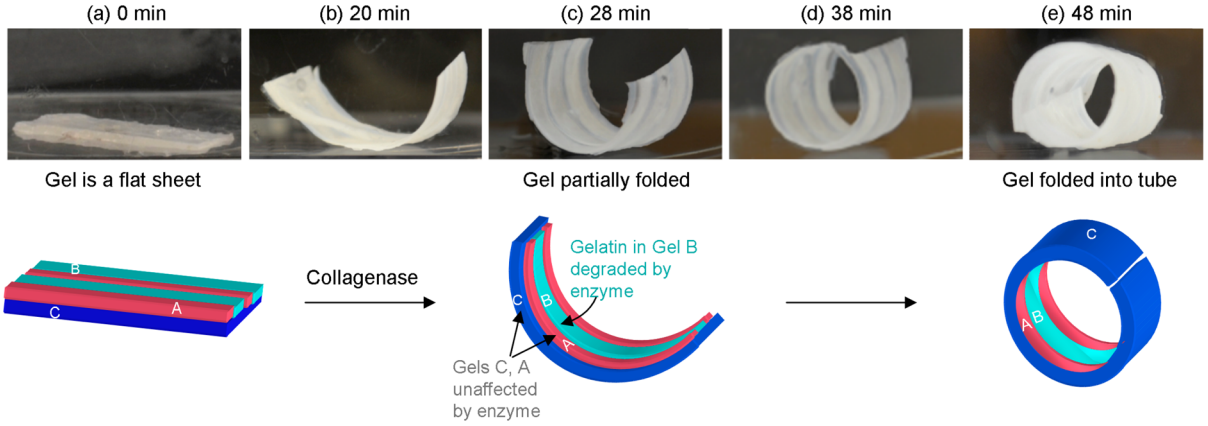

Figure 2. Folding of a hybrid-gel sheet upon exposure to collagenase enzyme. At t = 0, 50 U/mL of collagenase is added to the solution. Initially, the gel is flat, but with time it folds into a closed tube, as shown in photos in panels a to e that correspond to representative time points. The effect of the enzyme is to degrade the gelatin in Gel B, thereby softening the A/B layer. This causes the C layer to fold over the A/B layer, as shown by the schematics.

to UV light through the photomask (Figure 1a). The solution was gelled in the areas corresponding to the clear sections of the mask while the adjacent areas remained liquid. The ungelled liquid was washed off and then the pregel solution of Gel B was poured such that it filled the areas between the Gel A strips (Figure 1b). The Gel B precursor solution contained 10 wt % GelMA, 5 wt % PEGDMA and initiator. Upon UV irradiation, Gel B strips were formed between the Gel A strips (Figure 1c). Lastly, the precursor solution to Gel C (15 wt % NIPA, 3.5 wt % laponite, and initiator) was introduced above the A/B layer, and this was cross-linked by UV light to form the Gel C layer (Figure 1d). The final gel could be peeled away from the glass substrate and it had dimensions of 20 mm × 9 mm × 0.15 mm.

Spontaneous Folding of Gels in Response to Enzyme. We first studied the hybrid gel sheets in different media. The sheets remained flat in water, regardless of pH or salt concentration. They also remained flat in organic solvents such as ethanol or acetone. We then proceeded to study the effect of adding the enzyme collagenase to the aqueous solution surrounding a gel sheet. Collagenase is an enzyme from the class of matrix metalloproteinases (MMPs) that degrades certain portions of a gelatin gel into peptide fragments (note that gelatin is denatured collagen).[35] Specifically, collagenase looks for triple-helical segments where three individual gelatin/collagen chains bind together (native gelatin forms a gel at room temperature due to such physical junctions[36]). Although we have modified gelatin with methacrylates to make GelMA, this does not affect the chains' ability to form triple-helical junctions.[32,33] Thus, collagenase can still act on the GelMA chains in the Gel B portion of the sheet.

Figure 2 shows the response of a gel sheet to 50 units/mL of collagenase (type IV), which is introduced into the solution at t = 0. Over a period of 45 min, the flat sheet spontaneously curls up to form a closed tube of radius ~2 mm. The curling of the sheet is driven by the enzymatic degradation of GelMA, which causes the Gel B regions of the sheet to lose cross-links and hence become weaker. The mechanism for this shape change is discussed below. Note that the folding of the sheet occurs out-of-plane about an axis perpendicular to the length of the Gel A strips, as illustrated in Figure 2b–e. This is observed regardless of the aspect ratio of the sheet. The reason, as will be discussed below, is that the Gel A strips are relatively stiff. Therefore, folding along the axis perpendicular to the Gel A strips is more favorable than other alternatives.

We now quantify the kinetics of the shape change (flat sheet to folded tube) shown in Figure 2 using image analysis software. Specifically, when the gel is partially folded, we draw a circle that overlaps onto the bent gel (i.e., the gel forms an arc of this circle), as shown in Figure 3. This is termed the

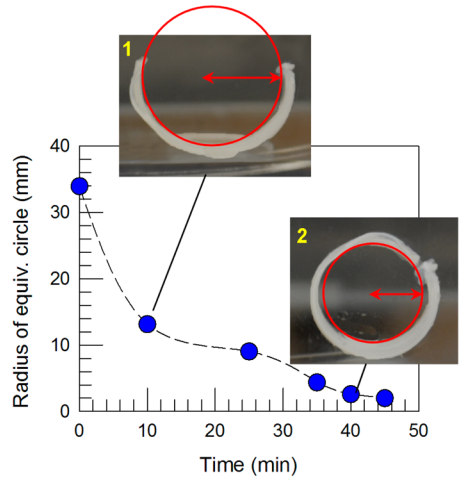

Figure 3. Kinetics of gel folding from flat sheet to tube. The folding shown in Figure 2 in response to 50 U/mL of collagenase is quantified by image analysis. At each time point, the image of the gel (side view) is mapped to an "equivalent circle", as shown in photos 1 and 2. The radius of this circle quantifies the extent of folding. As shown in the plot, this radius decreases with time, ultimately plateauing at the radius of the closed tube.

"equivalent circle", and the radius of this circle becomes smaller as the gel continues to bend and fold. Eventually, the gel is folded into a closed tube, i.e., there is no gap between the gel ends (photo 2). At this point, the equivalent circle coincides with the tube cross-section and the radius of this circle approaches 2 mm. Given further time, the tube continues to fold inward, i.e., the two ends start to overlap, but the radius of the tube does not decrease much. Even after 24 h in the enzyme solution, the tube remains folded at roughly the same radius.

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As a first step to proving that gel folding is indeed caused by the enzyme, we study the effect of enzyme concentration. An enzyme unit (U) is defined as the amount of enzyme that catalyzes the conversion of 1 micromole of substrate per minute. Here, we varied the concentration of the collagenase (type IV) enzyme from 60 to 0.75 U/mL. In each case, we used identical gel sheets and measured the time taken for a flat sheet to curl to form a closed tube (with a radius ~2 mm). As noted above, this "response time" was 45 min from Figures 2 and 3 for the case of collagenase at 50 U/mL. A plot of the response time vs enzyme concentration is shown in Figure 4. We see a

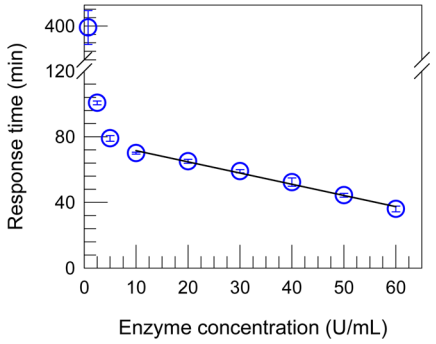

Figure 4. Effect of enzyme concentration on gel folding. The response time is defined as the time taken for a gel sheet to transform into a closed tube with a radius ~2 mm. The plot shows that this time decreases monotonically with enzyme concentration. A linear decrease is observed between 10 and 60 U/mL of enzyme. The error bars represent the standard deviation from 3 measurements.

roughly linear drop in response time as enzyme is increased in the range between 10 to 60 U/mL. This suggests a direct correlation between response time and reaction kinetics: i.e., the expected linear increase in reaction rates with enzyme concentration[30] is consistent with a linear decrease in response time. Below 10 U/mL, however, the response time increases in a nonlinear fashion as the enzyme content is decreased. This deviation from the linear trend may be because other factors, such as the mass transfer rates for enzyme into the gel, begin to play a role. In any case, it is worth noting that very low amounts of enzyme (<1 U/mL) are still able to induce the folding of the gel. Also, because the enzymatic degradation is irreversible, the enzyme-induced transition from a flat to folded gel is a one-time process.

Mechanism for Gel Folding. To understand folding better, we studied the mechanical and swelling properties of the three gels A, B, and C in the hybrid, both in the absence and presence of enzyme. For this, we made disks of each gel, 20 mm in diameter and 1 mm in thickness. These were studied under oscillatory shear in a rheometer and the elastic G' and viscous G'' moduli were measured as a function of frequency. As expected, all gels showed an elastic response, with the moduli being independent of frequency and $G' > G''$.[37] Thus, we can characterize each gel by its gel modulus, i.e., its value of G'. We find (Figure 5) that Gel A (PEGDA) is stiff (G' = 20 300 Pa), whereas Gel C (NIPA-LAP) is relatively weak (G' = 1,850 Pa). Both these gels are unaffected by the presence of collagenase. Gel B (GelMA-*co*-PEGDMA) is also weak at the start (G' = 1450 Pa). Moreover, its modulus decreases in the presence of collagenase. The plot in Figure 5 shows that, upon contact with 50 U/mL of enzyme, the G' of Gel B drops from 1450 to 750 Pa over the first 30 min and subsequently to 200 Pa over a period of 3 h. Over longer periods of time, the Gel B disk loses mass, and after about 6 h there is no trace of the gel in the solution (indicating complete degradation).

Regarding the swelling behavior, we first incubated each of the Gel A, B, and C disks in water in the absence of enzyme for 24 h. The disks were then weighed, then freeze-dried and weighed again. The swelling ratio Q was defined as the mass of the swollen disk divided by the mass of the dried disk. As shown in Figure 5, Gels A and B undergo negligble swelling in water ($Q \approx 4.7$ and 6.0, respectively) whereas Gel C swells considerably ($Q \approx 49$). Thus, there is a large swelling mismatch between the bottom (C) and top (A/B) layers of the hybrid gel. This swelling mismatch creates stresses within the film, giving it a propensity to fold, but the high stiffness of the A/B layer prevents folding. Upon incubation with enzyme, Gel B gets degraded, which sharply decreases its modulus (see above) and also causes a modest increase in its swelling ratio (Q increases from 6.0 to 9.1 after 30 min of contact with 50 U/mL of enzyme). Gels A and C remain unaffected by enzyme. Our experiments show that the key effect upon adding enzyme is

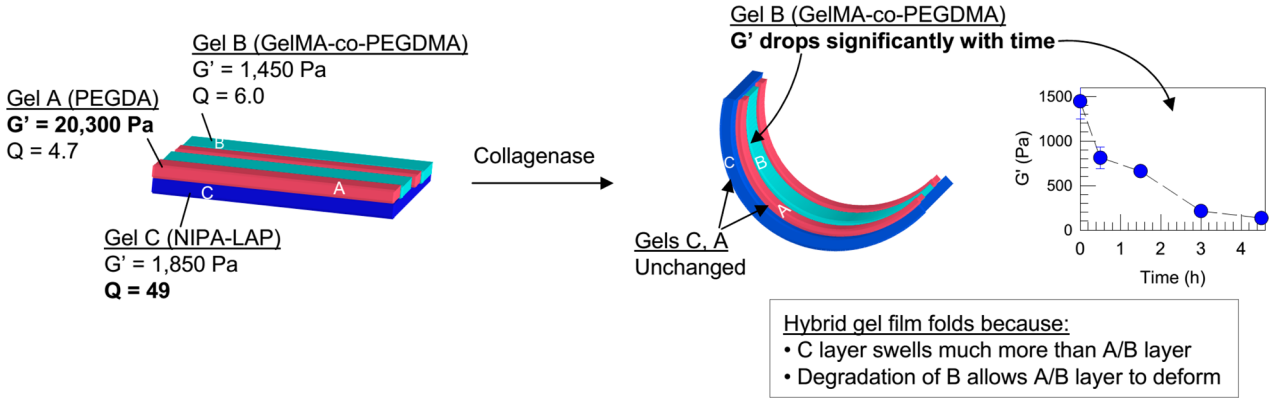

Figure 5. Mechanism for enzyme-induced gel folding. Data from two types of measurements are shown for the three individual gels in the hybrid when each of them is placed in water in the absence of enzyme: the elastic modulus G' (measure of gel stiffness) and the swelling ratio Q. The data reveal that Gel A is much stiffer than the others whereas Gel C swells much more than the others. The swelling mismatch between the C and A/B layers is the driving force for the folding, but it is opposed by the stiffness of the A/B layer. When exposed to enzyme, Gel B gets degraded, which drops its G' as time progresses, as shown by the plot on the right. Thus, the A/B layer becomes softer and more deformable, which allows the C layer to fold over it.

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the decrease in modulus of Gel B, which makes the A/B layer more pliable or compliant. As a result, the stresses created by the mismatch between the swollen C layer and the less swollen A/B layer are relieved when the sheet folds into a tube with the C layer outside and the A/B layer inside.[14,31]

The above reasoning is consistent with theories advanced for the bending of metallic strips as well as for the folding of gel sheets.[31,38] In the case of a metallic strip with a top and a bottom layer, a mismatch in thermal expansion coefficients between the two layers creates stresses when the strip is heated.[38] These stresses can be relieved by bending, provided the strip is not too mechanically rigid. The balance between these two factors is reflected in the Timoshenko equation.[38] For our hydrogel sheets, it is the difference in volume expansion (swelling) of the top and bottom layers that drives the folding. However, folding can occur only if the gel is mechanically compliant. Thus, the concept exploited here is to use an enzyme to turn a stiff layer into a compliant one, thereby enabling a transition from an unfolded to a folded gel. Support for the above mechanism is also provided by other experiments. For example, we studied an A/C design without Gel B (just two layers of Gel A and Gel C; see Figure S2a). In this case, no folding was observed due to the high modulus (G' = 20 300 Pa) of Gel A, which consists of 20 wt % PEGDA. Next, we lowered the concentration of PEGDA used to make Gel A, thereby decreasing the stiffness of the A layer. At 10 wt % PEGDA (G' = 830 Pa), the sheet slightly curled at its ends (Figure S2b), but did not fold further. At 5 wt % PEGDA (G' = 320 Pa), however, the sheet folded into a tube (Figure S2c). This confirms that folding is prevented by a stiff A layer whereas folding is enabled when the A layer is much more compliant.

Gel Folding in Cell Lysate. We have proven the ability of our hybrid gels to fold in the presence of specific biomolecules. Such a response could have applications in biological contexts; e.g., to signal the occurrence or proliferation of certain diseases. As a first step in evaluating such possibilities, we performed experiments with a class of murine fibroblast cells (L929). Fibroblasts are cells of the connective tissue that synthesize the extracellular matrix (ECM), a major component of which is collagen.[30,39] MMP enzymes that can degrade the collagen in the ECM are expected to be present in these cells (the ECM gets degraded and reformed during normal processes in the body like tissue remodeling as well as during diseased states like arthritis or cancer metastasis).[39,40] We therefore reasoned that the MMPs in L929 cells might plausibly act like the collagenase studied earlier and degrade the gelatin in Gel B. But would the concentration of MMPs in these cells be sufficient to drive folding of our hybrid gel within a reasonable time frame?

To test this, we made a suspension of L929 cells in buffer (10^6 cells/mL) and lysed the cells using a tip sonicator. The resulting lysate was a clear solution, which was then used for further studies. First, we incubated a disk of Gel B alone in the cell lysate. This gel degraded completely in a 24 h period, presumably due to breakdown of gelatin by the MMPs. In comparison, disks of Gel A and Gel C remained unaltered. Next, we placed our A/B/C hybrid gel sheet in the cell lysate (Figure 6). The gel converted from a flat sheet to a closed tube in about 6 h (photo in Figure S3a). To show that the result was due to the action of MMPs, we performed control experiments where we added MMP inhibitors to the cell lysate prior to placing the gel. Two inhibitors, copper sulfate and EDTA, were each tried at a 5 mM concentration. In both cases, the gel did not fold even after 24 h (photo in Figure S3b). Additional

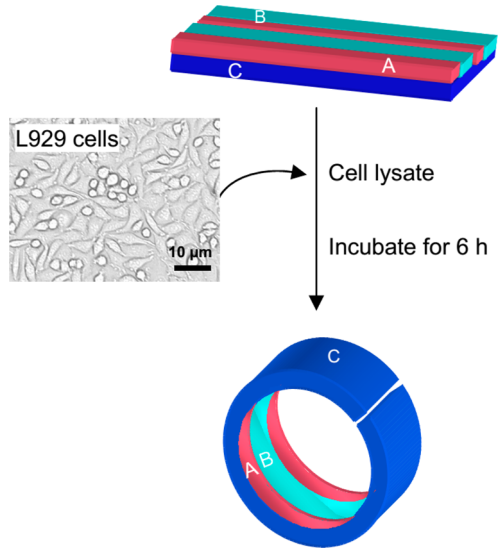

Figure 6. Gel folding induced by cell lysate. Mouse fibroblast (L929) cells were cultured and a suspension of the cells was lysed by sonication. This lysate was then introduced into a solution containing the A/B/C gel sheet. The flat gel folded into a tube in 6 h, and the folding is attributed to the presence of MMPs in the lysate (a class of enzymes that can degrade gelatin). The image shows a bright-field optical micrograph of the cells.

controls were also done: for example, the gel did not fold in just the cell growth media. Thus, our experiments strongly suggest that the folding is driven by MMPs from the L929 cells and that the typical intracellular (physiological) concentration of MMPs in these cells is sufficient to drive the macroscopic shape-change. From an extrapolation of the response time data in Figure 4, we estimate the concentration of MMPs in our lysate to be about 0.1 U/mL (which roughly translates to about 6 nM).

Venus Flytrap Analog. Although the folding of a single gel is interesting on its own, we can also incorporate a folding gel into other inert structures and thereby engineer other shape transitions. As an example, we have created an analog of the Venus fly trap based on hydrogels (Figure 7). Photos 1−3 show an actual Venus fly trap in action. As noted in the Introduction, the two adjacent leaves of the plant form a wedge-shaped compartment (photo 1). The leaves have hairs that are sensitive to touch, and so when an insect settles into the compartment and touches the hairs, the two leaves move together (photo 2) and completely close (photo 3), thereby entrapping the insect. The precise mechanism is postulated to involve induction of differential turgor pressure in the upper and lower portions of the leaves.[16−21] The leaves bend, and in the process store elastic energy. A sudden release of this elastic energy explains why the trap can close rapidly (in about 0.3 s).

Our mimic of the Venus flytrap is shown in Photo 4 and is illustrated schematically below the photo. Two oval "leaves" made of Gel A (PEGDA) are connected by a "hinge" or "stem" made from our usual hybrid gel (A/B strips on top, C on the bottom). The leaves are chosen to be rigid structures unaffected by enzyme, whereas the hinge is designed to fold on exposure to enzyme. The entire structure was created by photo-cross-linking and it remains a flat sheet in water. A load is placed on one leaf (photo 5). When collagenase is added to the water, it removes cross-links from Gel B, thereby driving the A/B/C hinge to fold. In turn, the leaf on top folds until it touches the

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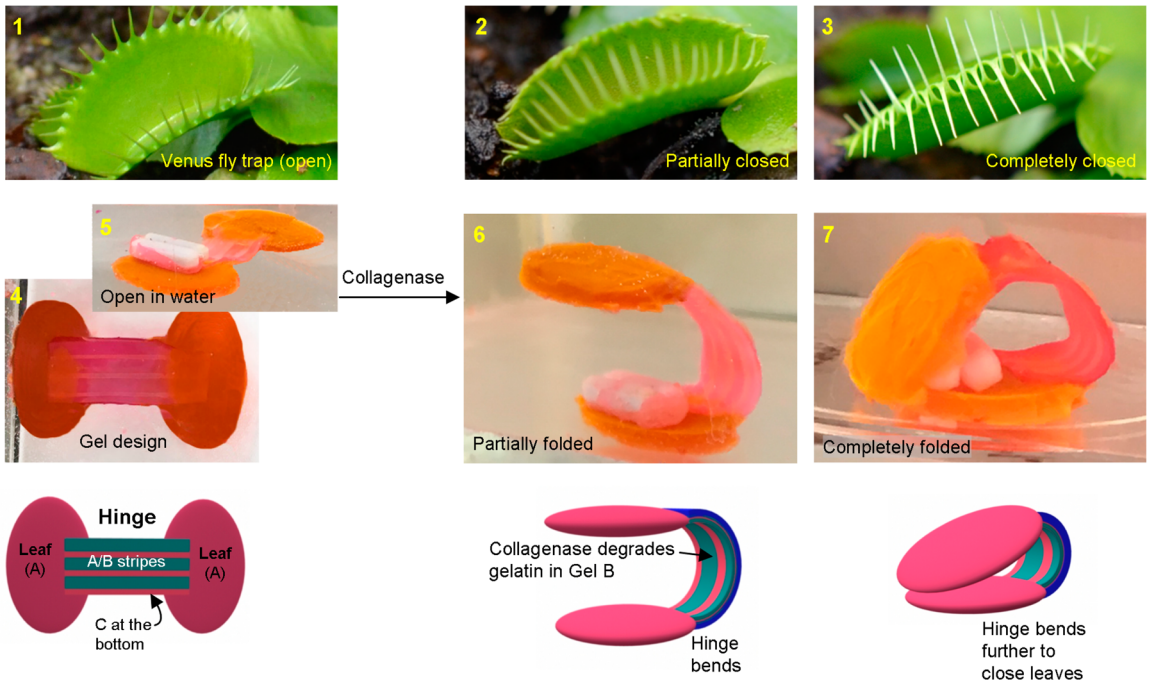

Figure 7. Hybrid gel that mimics the behavior of a Venus flytrap. Photos 1−3 show a Venus flytrap plant, which has a wedge-shaped cavity flanked by two leaves. When an insect falls in the cavity, the leaves close thereby capturing the insect for subsequent digestion. Photo 4 shows the design of our gel-based Venus fly trap mimic, and this is schematically shown below the photo. The rectangular hybrid gel from Figure 1 is affixed as a hinge to two flat panels ("leaves") made of Gel A. The hinge has dimensions of 20 mm × 9 mm × 0.15 mm, whereas each leaf is a disc with dimensions of 20 mm × 15 mm × 1 mm (thus the leaves are much thicker than the hinge). Photo 5 shows that the structure is flat in water. A load (two magnetic stir bars) is placed on one of the leaves. When exposed to collagenase, the hinge bends, and thus the leaves close around the load, as shown in photos 6 and 7. The schematics show the directionality of bending: in the hinge, the C layer is outward whereas the A/B layer is inward.

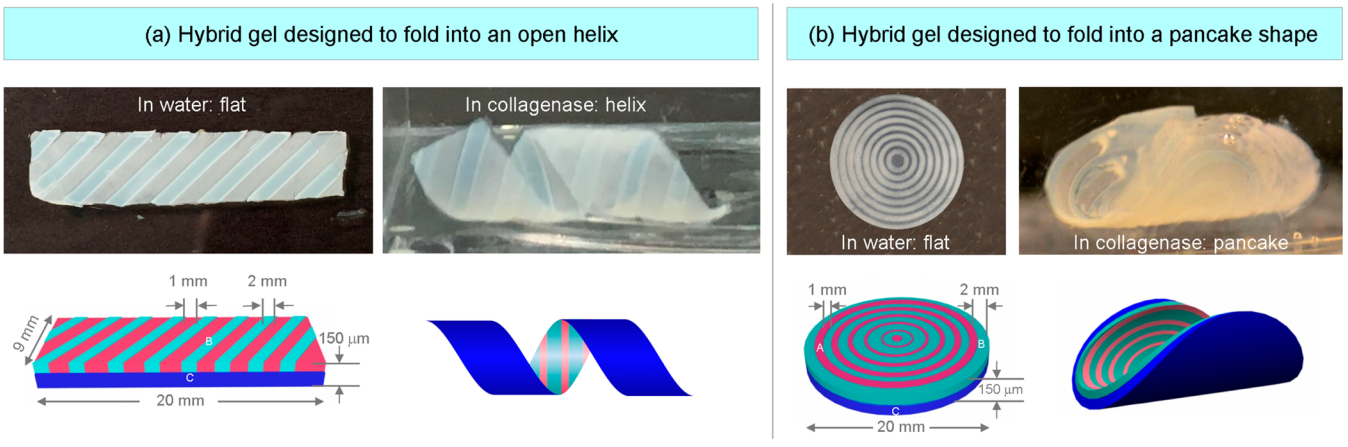

Figure 8. Designed hybrid gels that fold into different shapes. Both designs feature three component gels (A, B, and C), as in Figure 1. In each case, photos of the gel in water and in collagenase solution are shown. Schematics of the design and the folded structure are also shown. (a) The design has A/B strips on top at an angle of 45° with respect to the bottom C layer. This gel is a flat sheet in water, but folds into an open, right-handed helix when exposed to collagenase. (b) A design with concentric circles of A and B in the top layer and C in the bottom layer. In this case, the gel folds into a pancake shape in the presence of collagenase.

bottom leaf (photos 6 and 7), thereby enclosing the load. The time for the leaves to fold completely is about 50 min for 50 U/mL of enzyme, which is close to the time measured for the folding of a single gel sheet under the same conditions (Figure 3). Thus, we have mimicked the shape change of the Venus flytrap, albeit with hydrogels.

Other Shape Changes. Our gel design can be adapted to achieve other transitions in shape upon exposure to enzyme. In some cases, we can predict the final shape *a priori*. An example is the A/B/C design in Figure 8a. This design is similar to that in Figure 1 but with one difference: the A/B strips in the top layer are at an angle of 45° with respect to the bottom C layer. The overall dimensions are similar to those in Figure 1, with the Gel A strips being 1 mm wide and the Gel B strips being 2 mm wide. This gel is initially flat in water and it is then exposed to collagenase. We noted previously that folding occurs along an axis perpendicular to the stiff Gel A strips. Here, because the strips are at an angle, we anticipated that folding would lead to a helix rather than a closed tube. Indeed, the folded structure is seen to be an open, right-handed helix in Figure 8a. Similar

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folding of flat gel strips into helices has been observed in a few studies.[14,15] It is also observed with plant seedpods,[23] and there also the folding occurs perpendicular to the direction of stiff cellulose fibrils that are embedded at an angle within the pods.

A second design of a folding gel is shown in Figure 8b. This is an A/B/C design in the form of a disc rather than a rectangular sheet. As before, the bottom layer is Gel C whereas the top layer has alternating concentric circles of Gels A and B. The Gel A circles are 1 mm thick whereas the Gel B circles are 2 mm thick. In this concentric design, there is no preferred axis for the gel to fold. Instead, when the flat gel is placed in collagenase solution, it transforms into the shape of a folded pancake (Figure 8b). The swollen Gel C layer is on the bottom whereas the A/B layer is on the top. A gel patterned with a series of concentric circles was recently studied by Kumacheva et al.,[13] and in that case the folded shape was similar, but with negative curvature in the middle, i.e., resembling a saddle rather than a pancake. More detailed analysis of the above geometry is beyond the scope of the present paper. In closing, we reiterate the versatility of our system, which permits a variety of shape changes to be induced by enzyme with the same underlying materials, but with different designs.

CONCLUSIONS

We have designed gels that undergo a transformation in shape when exposed to low concentrations of a specific enzyme. The typical transformation is from a flat sheet to a folded tube. Our motivation in creating these gels was to expand the range of triggers for gel systems beyond the common ones like temperature and pH. Moreover, our approach to accomplishing these objectives focused on physical, rather than chemical design, i.e., without resorting to extensive synthetic chemistry.

Toward this goal, we have put forward a design for "hybrid" gels, involving three individual gels with distinct properties. Gel C swells highly in water compared to Gels A and B, whereas Gel A is much stiffer than the rest. Gel B is the one that is sensitive to enzymes. All gels are based on common, commercially available monomers, with the exception of Gel B, for which we synthesized a methacrylated version of gelatin. We integrated the three gels together into thin-films using UV polymerization through homemade photomasks. The base design involves an A/B layer on top of a C layer. When placed in water, the sheet is initially flat despite the swelling mismatch between the layers. When collagenase enzyme is added, it cleaves the gelatin chains in Gel B, thus reducing the stiffness of the A/B layer. This allows the swollen C layer to fold over the A/B layer. The time to fold can be tuned by the concentration of enzyme.

The versatility of our underlying A/B/C design is shown by modifying the physical pattern to achieve other shape changes. For example, we can engineer the folding of flat sheets into helices or pancake shapes, instead of tubes. We have also constructed a structure reminisent of the Venus flytrap. This structure transforms from an open to closed state upon exposure to enzyme, with the "leaves" on either end trapping objects in the middle. In summary, our study highlights the possibility of creating complex designs using hydrogels that could more closely mimic structures seen in nature, both in terms of their form as well as their function. Hybrid hydrogels such as the ones demonstrated here could have potential applications in tissue engineering, soft robotics and biosensing.

MATERIALS AND METHODS

Materials. The monomers PEGDA (535 Da), PEGDMA (330 Da), and NIPA, and the reagents methacrylic anhydride (94%), octadecyl-trichlorosilane (OTS) (≥90%), anhydrous copper(II) sulfate, EDTA, and toluene were purchased from Sigma-Aldrich. Ethanol was from Pharmco-Aaper whereas the UV initiator Irgacure 2959 was from BASF. The inorganic clay laponite-XLG was obtained from Southern Clay Products. Phosphate buffered saline (PBS) was was also obtained from Sigma-Aldrich. The collagenase type IV enzyme (305 U/g) was from Worthington Biochemical Corp. NIPA was recrystallized using hexane as a solvent to remove any inhibitor. All other chemicals were used as received. Ultrapure deionized (DI) water from a Millipore system was used for all experiments.

Synthesis and Characterization of Gelatin Methacrylate (GelMA). GelMA was prepared using the procedure described by Nichol et al.[33] Briefly, 10% (w/v) gelatin was dissolved in PBS by heating to 65 °C. 5% (v/v) methacrylic anhydride was then added and the temperature was maintained at 65 °C for 2 h. Afterward, the solution was diluted with 5X PBS to stop the reaction. The solution was dialyzed against DI water using a membrane with a 12−14 kDa cutoff at 40 °C for 1 week. The purified solution was then lyophilized and the dry powder was stored in a freezer. To verify attachment of methacrylate groups to gelatin, ^{1}H NMR spectra were collected on GelMA in deuterium oxide at 65 °C using a Bruker AVANCE 600 Hz spectrometer.

Preparation of Hybrid Gels. All monomers were dissolved in DI water through which nitrogen gas had been bubbled for 30 min to remove dissolved oxygen. In each case, 0.5% (w/v) of Irgacure 2959 was added as the UV initiator. For Gel A, the pregel solution contained 20 wt % of PEGDA. For Gel B, the pregel solution was composed of 10 wt % of lyophilized GelMA and 5 wt % of PEGDMA. For Gel C, 1 M of NIPA was first dissolved and then 3.5 wt % of laponite was slowly added to the solution while stirring to avoid clumping.

Glass slides (75 mm × 25 mm) coated with OTS were used in preparing gels. The OTS coating ensures that the gels can be easily detached following UV polymerization. For this, slides were rinsed with ethanol and then immersed in a solution of 0.2% OTS in toluene for 15 min. The slides were then rinsed several times with toluene to remove excess OTS, then dried by placing in an oven at 90 °C for 1 h.

A reaction cell was made using two glass slides separated by double-sided tape on two ends. The Gel A precursor solution was introduced into the cell and UV polymerized through a photomask (Figure 1a). All photomasks were designed on Adobe Illustrator CS6 and printed on transparencies using a Brother Multifunction Laser Printer. The UV light source was a Xenon RC500 lamp which emitted radiation over the entire UV spectrum. Following polymerization, unreacted monomer was rinsed off the reaction cell using DI water. Then the Gel B precursor was introduced (Figure 1b) and polymerized. Next, another layer of double-sided tape was added to adjust the height of the reaction cell. Finally, Gel C precursor was introduced into the cell and polymerized by UV light (Figure 1d). The hybrid gel was then peeled off the reaction cell, and cut to the dimensions shown in Figure 1e. Gels were soaked in DI water overnight to remove unreacted monomer. For preparing the other gel designs such as the Venus flytrap analog, the same procedure was used, but with different photomasks.

Rheological Studies. Dynamic rheological experiments were performed on an AR2000 stress-controlled rheometer (TA Instruments). Experiments were done at 25 °C using 20 mm parallel plates on gel disks of diameter 20 mm and thickness 1 mm. A solvent trap was used to minimize drying of the samples. Frequency sweeps were conducted within the linear viscoelastic regime of the sample, determined separately from strain-sweep experiments.

Swelling Measurements. Disks of Gels A, B, and C, were separately made with a diameter of 20 mm and a thickness of 1 mm. The disks were immersed in DI water and allowed to swell for 24 h. Next, the disks were blotted dry using a Kimwipe and weighed. Afterward, the disks were lyophilized and weighed again. The swelling

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ratio Q was calculated as the ratio between the swollen and dry weights.

Cell Culture And Cell Lysate. Mouse fibroblast cells (L929) were purchased from ATCC, as was Eagle's modified essential medium (EMEM). Penicillin−streptomycin (Pen-Strep), fetal bovine serum (FBS), and trypsin were purchased from Gibco. For L929 cell culture, EMEM was supplemented with 10% FBS and 1% Pen-Strep. The cells were cultured in T-75 flasks in a 37 °C incubator with 5% CO_2. Cells were passaged approximately twice a week and media was exchanged every 2 days. To obtain cell lysate, the cultured cells were typsinized and resuspended in PBS at a concentration of 10^6 cells/mL. The solution was then sonicated on ice for a total time of 1 min at a power of 180 W (10 s of sonication followed by 10 s of rest). Following sonication, the clear lysate left behind was used in further studies.

■ ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/acsami.6b05024.

Data on the synthesis and characterization of gelatin methacrylate (Figure S1), the folding of A/C bilayer gels (Figure S2), and the folding of gels in cell lysate (Figure S3) (PDF).

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Notes

The authors declare no competing financial interest.

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Supporting Information for:

Enzyme-Triggered Folding of Hydrogels: Towards a Mimic of the Venus Flytrap

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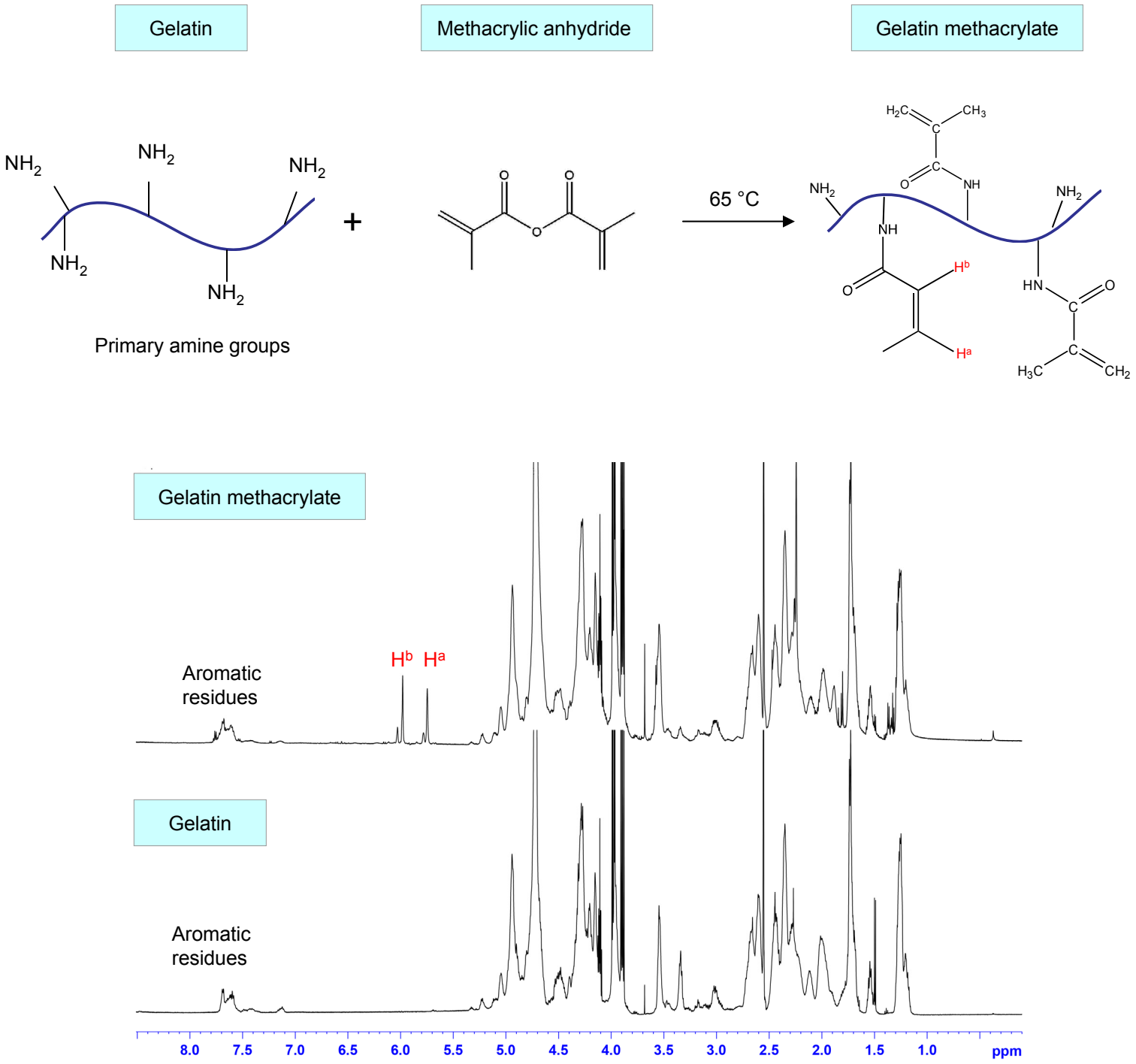

Figure S1. Synthesis of gelatin methacrylate and analysis by ^{1}H NMR. The reaction scheme (as per Ref. 28) is shown on the top. Lysine and arginine residues in gelatin are converted to methacrylate groups by reaction with methacrylic anhydride. NMR spectra are compared for the parent and synthesized polymer. The peaks H^a and H^b at 5.6 ppm confirm the addition of methacrylate groups to gelatin. The integrated intensities due to the aromatic residues, H^a and H^b were used to estimate the degree of functionalization (i.e., the ratio of the number of amino groups modified with methacrylate to the initial number of amino groups), as described in Ref. 27. Here, the degree of functionalization is about 60%.

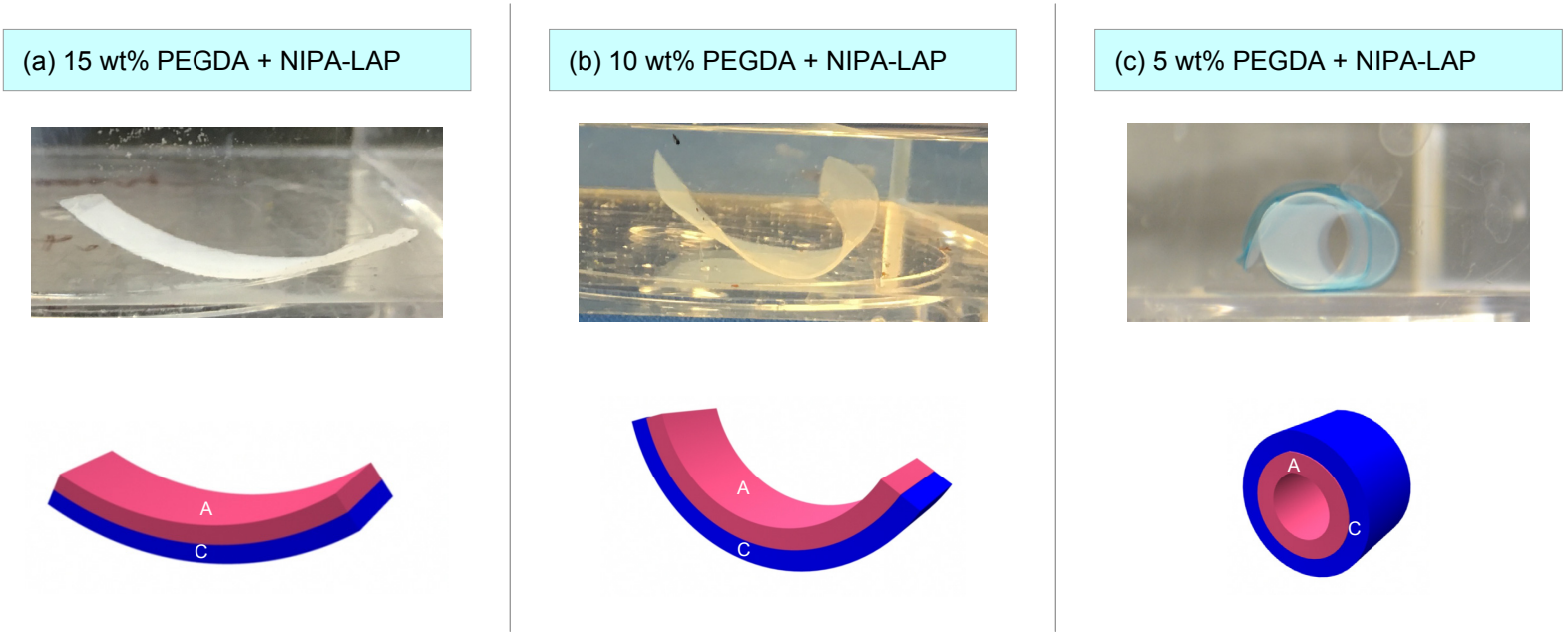

Figure S2. Folding behavior of bilayers of Gel A (PEGDA) and Gel C (NIPA-LAP). The dimensions of each gel was maintained at 20 mm x 9 mm x 0.15 mm. The NIPA-LAP is identical to that in Figures 2-5. The concentration of PEGDA was decreased from (a) to (c). Note that folding of the flat sheet into a tube is observed only when the PEGDA is reduced to 5 wt%. At higher concentrations of PEGDA, the stiffness of the A layer opposes folding.

Figure S3. Visual observations of hybrid gels folding in cell lysate. Mouse fibroblasts (L929) cells were cultured and the cell lysate was obtained. The hybrid gels were then incubated in the cell lysate. (a) The initial flat sheet folded to form a tube in 6 h due to the presence of MMPs in the lysate. (b) As a control, we added 5 mM EDTA (an inhibitor of MMPs) to the lysate. In this medium, the gel sheet remained flat.