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Multilayer tubes that constrict, dilate, and curl in response to stimuli†

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Tubular structures in nature have the ability to respond to their environment—for example, blood vessels can constrict or dilate, thereby regulating flow velocity and blood pressure. These tubes have multiple concentric layers, with each layer having a distinct composition and properties. Inspired by such natural structures, we have synthesized responsive multilayer tubes in the laboratory without resorting to complex equipment such as a 3-D printer. Each layer of our tubes is a polymer gel formed by free-radical polymerization of water-soluble monomers. We can precisely control the inner diameter of the tube, the number of layers in the tube wall, and the thickness and chemistry of each layer. Tubes synthesized in this manner are robust, flexible, and stretchable. Moreover, our technique allows us to incorporate stimuli-responsive polymers into *distinct regions* of these tubes, and the resulting tubes can change their shape in response to external stimuli such as pH or temperature. In the case of *laterally patterned* tubes, the tube can be made to constrict or dilate over a particular segment—a behavior that is reminiscent of blood vessels. In the case of *longitudinally patterned* tubes, a straight tube can be induced to systematically curl into a coil. The versatility of our technique is further shown by constructing complex tubular architectures, including branched networks. On the whole, the polymeric tubes shown in this paper exhibit remarkable properties that cannot be realized by other techniques. Such tubes could find utility in biomedical engineering to construct anatomically realistic mimics of various tissues.

Introduction

Polymer hydrogels that change their shape have been investigated by many groups over the recent years.[1–7] The inspiration for making such gels has come from nature, where shape-changes are routinely observed in structures such as plant leaves or seed pods. For example, the Venus flytrap is an insectivorous plant with its leaves arranged as a wedge.[2,5] When an insect lands in the wedge, the leaves fold and envelop the insect, which is then digested. Polymeric analogs of the above are typically in the form of rectangular gel sheets, which are induced to fold (curl) in response to either external stimuli like temperature or pH,[1–4] or upon addition of solutes like enzymes or multivalent ions.[5,7] To induce such folding, the gel sheet is fabricated to be a sandwich of multiple layers. The sheet will fold if one layer of the sandwich swells more in water compared to the adjoining layer; this difference in swelling leads to mechanical stresses that drive the folding. In the case of the Venus flytrap, a similar mechanism is responsible for the folding of the leaves, *i.e.*, the leaves transduce the arrival of the insect into a difference in turgor pressure between adjoining layers of leaf tissue.[2,5]

Multilayered materials are also pervasive in nature in geometries other than flat sheets. Spherical structures with multiple layers are well-known, including seeds, eggs, and vegetables like the onion.[8,9] In addition, *tubes* (hollow cylinders) with multilayered walls are commonly found in nature, and these are particularly relevant to this paper. Specifically, the vessels transporting fluids and nutrients in the body are all multilayer tubes.[10] For example, veins and arteries (see Fig. S1 in the ESI†) have three main layers in their walls.[10,11] The intestines, both the duodenum and the colon, also have multilayered walls. Each individual layer of these tubes has a distinct chemical composition and nanostructure. In turn, each layer has different properties and confers distinct functions to the overall structure. (Indeed, biology is full of examples where individual parts are integrated into a whole that is greater than those parts.) Note that all layers in biological tubes are gel-like—*i.e.*, they are soft, elastic solids with significant (>50%) water content. One of the remarkable properties of blood vessels is their ability to constrict (*i.e.*, narrow their inner diameter) or dilate (*i.e.*, expand their diameter) in response to systemic or local variables. This is how the body regulates blood pressure and flow rate. Molecules that can induce dilation of blood vessels include nitric oxide (NO), epinephrine, and adenosine.[10,11]

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Is it possible to create synthetic polymer tubes that can mimic the remarkable architecture or properties of biological tubes? That is, for instance, could one fabricate a tube with a wall composed of *multiple, concentric layers* (with each layer being a different polymer gel)? Or could one induce a tube to undergo a shape change akin to blood vessels—*i.e.*, to *constrict or dilate spontaneously*—in response to external stimuli? These are some of the intriguing challenges that motivated the research described in this paper. In the literature, one can find many attempts at creating polymer tubes, and in many cases the motivation has been to mimic the structure of blood vessels or intestinal tubes. However, such structures have usually been made by complex, labor-intensive techniques such as 3-D printing,[12–16] laser ablation,[17] or electrospinning[18,19] that require specialized equipment and expertise. Others have tried to generate tubes by starting with flat sheets and then inducing these to fold or roll up (*via* pH, temperature, *etc.*); however, these tubes are still open at their edges, *i.e.*, they do not have structural integrity.[6,7] In all these previous studies with tubes, we have not found any example of a tube capable of changing its diameter as described above.

Here, we describe a new approach for creating multilayer tubes through an 'inside-out' polymerization technique.[8] This approach, which is illustrated in Fig. 1 and described below, allows us to grow successive concentric layers of polymer hydrogels around a cylindrical core. The technique is very simple, yet versatile. No specialized equipment or apparatus like those for electrospinning or 3-D printing are necessary. All polymers are based on commercially available monomers and crosslinkers, and the entire synthesis is carried out under mild, aqueous conditions. After the synthesis, the core is removed by heat (or other means) to yield a hollow tube that is robust and flexible. We can control the tube diameter as well as the thickness and composition of each layer in the tube wall. We can also use this approach to create tubes with responsive properties; this is done by incorporating specific polymers into an entire layer or into distinct regions of the tubes. Thereby, we demonstrate a tube that *shrinks or expands over a discrete segment* when aqueous fluid at a given temperature or pH is passed through it. This is reminiscent of blood vessels that constrict or dilate.[10,11] Overall, our approach is a straightforward way to make tubes in the laboratory and moreover, it can be used to create new tubular structures that cannot be accessed by other methods. We believe the simplicity and versatility of this approach will make it attractive for a variety of applications, especially in biomedical or tissue engineering.

Results and discussion

Synthesis of single-layer tubes

Our approach to generate a single-layer polymer tube is shown schematically in Fig. 1. First, we synthesize a cylindrical template of the biopolymer agar in a mold. Templates based on other biopolymers like alginate, chitosan, or gelatin can also be used, but agar is particularly convenient for our purpose due to its thermoresponsive properties.[20,21] Agar dissolves in hot water (~90 °C), and upon cooling to room temperature it sets into a gel. When the gel is reheated to 90 °C, it melts into a solution. Here, we introduce a hot agar (5% by weight) solution into Tygon® tubing of a pre-determined diameter and allow the solution to cool into a gel at ambient temperature. The agar cylinder is then forced out of the tubing by injecting DI water. This cylinder is then cut into pieces of desired length. Fig. S2 (ESI†) shows agar cylinders of different diameters made in this manner.

Next, the agar cylinder (Fig. 1a) is incubated in a solution containing a water soluble free-radical initiator, typically ammonium persulfate (APS). This is done for ~15 min, which

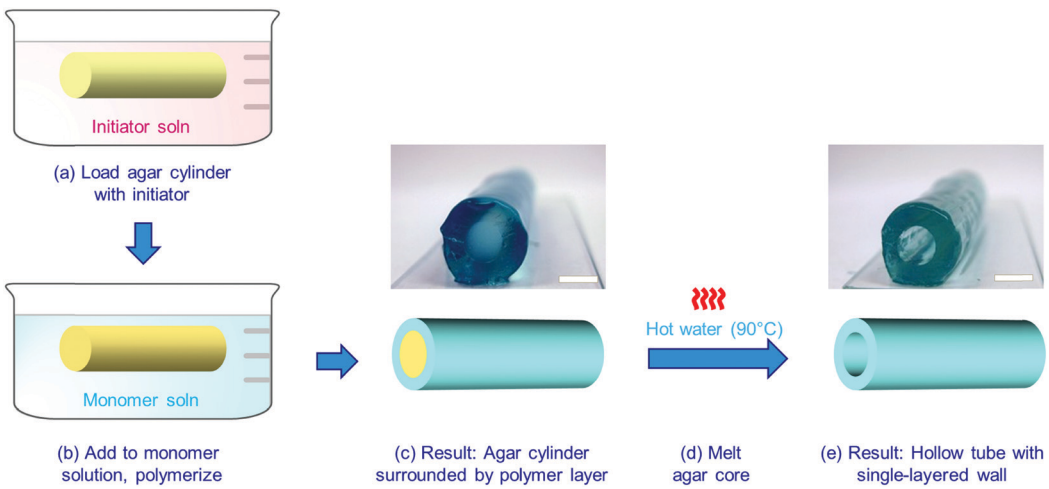

Fig. 1 Synthesis of a single-layer tube. (a) An agar cylinder of desired length and diameter is immersed in a solution of a free-radical initiator. (b) The initiator-laden cylinder is transferred to a solution of monomers. (c) The initiator diffuses outward and induces a layer (shell) of polymer to form around the core cylinder (in the photo, the cylinder end is cut to show the core and shell). (d) To yield a hollow tube, the ends are cut off and the structure is placed in hot water at ~90 °C, whereupon the agar core melts (gel to sol transition) and is removed. (e) The final result is a hollow tube with a wall composed of a single layer of polymer gel, as shown by the schematic and photo. Scale bars in (c) and (e) are 4 mm.

is sufficient time for the APS to diffuse into the entire cylinder. The initiator-laden cylinder is then transferred into a solution containing a monomer (*e.g.*, an acrylamide), a crosslinker, an accelerant, and a thickener such as xanthan gum (XG) (Fig. 1b).[8] The high viscosity of the solution (~70 Pa s for 0.5% XG) ensures that the cylinder remains suspended in it. The initiator then diffuses out into the surrounding solution and induces the growth of a polymer layer around the core cylinder (Fig. 1c). Once a layer of desired thickness has formed (typically in 10 to 20 min), the structure is removed and washed. Then the hemispherical caps at the ends are cut off with a razor blade to reveal the agar core. The whole structure is then placed in hot water at 90 °C for ~20 min to melt away the agar (Fig. 1d). This results in a hollow tube with a polymeric wall of desired composition and thickness (Fig. 1e).

Tubes synthesized by this technique can be created with a variety of lumen (inner) diameters and wall thicknesses. Fig. 2a displays tubes with inner diameters (IDs) ranging from 0.6 to 4.5 mm, which are made by varying the diameter of the agar core cylinders. All have a wall that is a gel-network of *N*,*N*′-dimethylacrylamide (DMAA) crosslinked with *N*,*N*′-methylenebis(acrylamide) (BIS). The wall thicknesses of the tubes in Fig. 2a are ~1 mm. This thickness can be controlled by varying either the initiator concentration (more initiator results in thicker layers[8]) or the polymerization time (layer thickness grows with time, see Fig. 3 below). Regarding the removal of the core, if this has to be done under milder conditions (*e.g.*, for biomedical applications), then a core of gelatin gel can be used instead of the agar gel. The gelatin core can be degraded either by heating to 35 °C or at room temperature by using enzymes such as collagenase.[22] In many cases, we have also found that the agar core can be pushed out simply by applying mechanical force on one end.

To visualize tubes more clearly, we incorporated the nanoclay LAPONITE® (LAP) into our synthesis scheme (see Fig. S3 in the ESI†). LAP is a disk-shaped nanoparticle that serves as a chemical crosslinker for growing polymer chains; moreover, the anionic faces of LAP disks have a strong affinity for cationic dyes.[23,24] We typically use a monomer composition of 10% monomer (*e.g.*, DMAA), 0.05% BIS and 3% LAP. Thus, LAP particles are the main chemical crosslinkers for the polymer chains, and the advantage is that gels crosslinked by LAP are more flexible and robust than those crosslinked by BIS.[24] The rest of the procedure is the same as before: the agar cylinder is placed in the above solution, and a layer of DMAA–LAP gel forms around it in 15 min. A photo of this tube after removal of the core is shown in Fig. S3a (ESI†). The tube wall is nearly transparent and colorless. This tube is then placed in a solution of the cationic dye, methylene blue (MB) at a concentration of 10 μM. The MB molecules adsorb on the faces of LAP disks, turning the tube wall blue. We can impart either a light blue color (Fig. S3b, ESI†) or a dark blue color (Fig. S3c, ESI†) to the tube wall depending on the time for which the tube is soaked in MB. Note that MB adsorption is strong and irreversible—so the dye does not subsequently diffuse out of the tube when it is placed in water.[23] Using this method, we can selectively 'stain' layers of a given tube, and we will use this staining technique throughout this paper.

Photos of a tube with a DMAA–LAP layer are shown in Fig. 2b–d. The tube has a light blue color, which is achieved

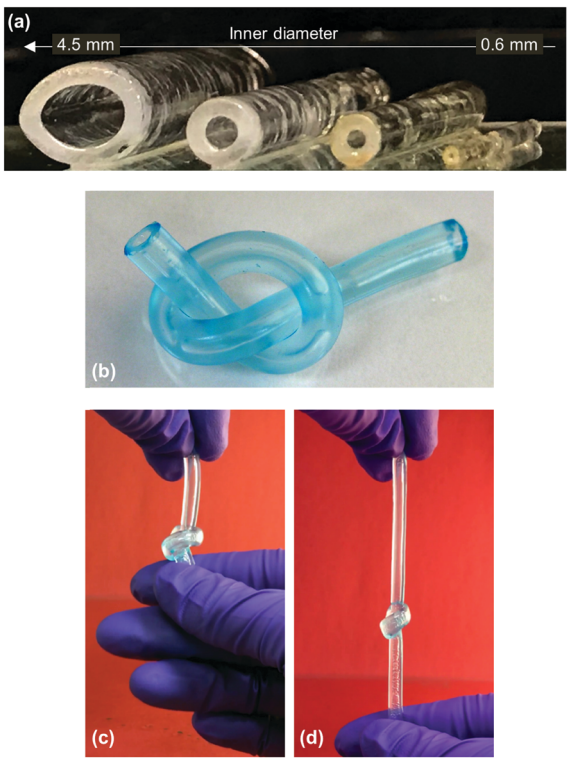

Fig. 2 Tube dimensions and mechanical properties. (a) Tubes with lumen (inner) diameters ranging from 0.6 to 4.5 mm are shown. In all cases, the tube wall is a single-layer DMAA–BIS gel (~1 mm thick). The photos in (b) to (d) show a tube with a single-layer (DMAA–LAP) wall. The tube is flexible and robust enough to be bent and tied into a knot. In (d), the knotted tube is stretched to ~150% of its initial length without breaking.

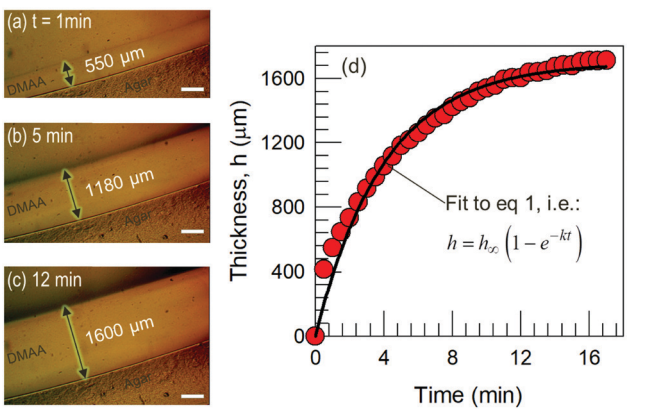

Fig. 3 Kinetics of polymer layer growth. An agar cylinder loaded with initiator is placed at t = 0 in a solution of the monomer DMAA and the crosslinker BIS. Images at various times during the polymerization in (a), (b) and (c) reveal the outward growth of the polymer layer from the agar core. Scale bars in the images are 500 μm. In (d), the thickness h of the polymer layer is plotted as a function of time t, and the curve through the data is a fit to eqn (1).

by soaking in MB dye for just 1–2 min. The photos indicate that the tubes are flexible, stretchable and highly robust. Fig. 2c and d show that the tube can be tied into a knot, and the knotted tube can be stretched without rupture. The mechanical robustness is especially evident in tubes made with LAP as the crosslinker (as opposed to BIS). This is a well-known property of LAP-crosslinked gels, and it is believed to be due to the increased length of polymer chain segments between crosslinking junctions.[24] Mechanical properties under tension were measured for a tube with a layer of *N*-isopropylacrylamide (NIPA) crosslinked by LAP. The results (Fig. S4, ESI†) show that the tube has a Young's modulus of 20 kPa and an elongation at break of 750%. Thus, the tubes are robust enough to support the steady flow of liquids through their lumen. This includes water with included additives (see below) as well as more viscous liquids like blood.

Kinetics of layer growth

The 'inside-out' growth of a polymer layer around the core cylinder can be visualized in real-time by optical microscopy. For this experiment, an agar cylinder (1.2 mm diameter) containing 15 mg mL^{-1} APS was placed in a monomer solution (10% DMAA, 0.34% BIS). A movie of the growing layer was captured at room temperature, and representative stills from the movie are shown in Fig. 3a–c. The layer thickness h from the images is plotted as a function of time in Fig. 3d. The data reveal rapid growth of the layer, reaching a thickness of 550 μm after 1 min (Fig. 3a) and saturating at a thickness h_∞ of 1600 μm by about 15 min (Fig. 3c). This saturation value does not increase further if the polymerization is continued for several hours, which suggests that a steady-state is reached when the initiator in the core is completely depleted. We fit the $h(t)$ data to the following equation:[8,25]

$$h = h_\infty(1 - e^{-kt}) \quad (1)$$

The parameter k in the equation is an effective rate constant and its value for the fitted curve in Fig. 3d is $k = 0.21\ min^{-1}$. The curve does deviate from the data over the initial 2 min of layer growth, but thereafter it fits the data well. Note that k accounts for both the diffusion of initiator into the monomer solution as well the kinetics of polymerization, with the two steps occurring in series.[25,26]

Tubes with lateral patterns

We now describe the synthesis of tubes with patterned regions of different polymers in the same material, of which one or more polymers could be responsive to stimuli. First, we show how to create patterns in the lateral direction (Fig. 4), *i.e.*, where various lateral segments of a tube are made with different polymer gels. The synthesis scheme is the same as before, but with more than one monomer solution in the container. Fig. 4a illustrates lateral patterning into three zones, each with different monomers. Here, we are using an approach that had previously been developed in our laboratory to synthesize hybrid (solid) gels with distinct segments. The underlying concept behind this method is that highly viscous solutions will not mix.[24,27,28] To adapt this idea here, our monomer solutions are made viscous by adding either 0.5% XG or 3% LAP. In a rectangular trough, we place two glass slides vertically at discrete points (Fig. 4a). This divides the trough into chambers, and the three viscous monomer solutions are added to the three chambers. The glass slides are then removed, whereupon the adjacent solutions do not mix due to their high viscosity (there will only be slight mixing at the interfaces). An agar cylinder loaded with initiator is then positioned perpendicularly across the three monomer solutions and is left to polymerize. After polymerization, the agar core is melted away to yield the patterned tube (Fig. 4a).

One example of a tube synthesized by the above method is shown in Fig. 4b. This is a laterally patterned tube where the middle segment is a gel of the thermoresponsive polymer NIPA and the end segments are gels of DMAA (all layers are crosslinked with BIS). The initial photo of this DMAA–NIPA–DMAA tube reveals a smooth structure with no obvious demarcation of the three zones. The differences emerge upon heating. Above its lower critical solution temperature (LCST) of ~32 °C, NIPA gels becomes opaque and shrink.[27,29] Correspondingly, here, we observe that the NIPA segment of the tube *constricts* above 32 °C. Both the outer (OD) and inner diameter (ID) of the tube are reduced (inset images in Fig. 4b): the OD from 5.0 to 3.6 mm and the ID from 2.1 to 1.0 mm. This change in diameter is rapid (takes less than a minute) and reversible, *i.e.*, the original sizes are restored upon cooling. Similar changes in diameter are shown for a NIPA-only tube in Fig. S5 (ESI†).

Next, we show a laterally patterned tube in which one segment is responsive to the pH of the solution (Fig. 4c). Here, the middle segment is a gel made by copolymerizing sodium acrylate (SA) and DMAA in a 20 : 80 molar ratio, while the end segments are gels of DMAA alone (all layers are crosslinked with BIS). The middle segment is labeled SA and the overall tube is labeled DMAA–SA–DMAA. While DMAA is nonionic, the SA segment can be anionic or nonionic depending on the pH.[27,29] That is, when the carboxylates on SA are ionized, *i.e.*, at pH > 7, the SA chains become anionic and as a result, its gel swells appreciably.[29] At low pH, the charge is lost and therefore the SA gel swells less. Accordingly, we study our tube in water at a pH above 7 and at a low pH of 3. At the low pH, all segments of the tube have the same diameter. At pH > 7, the SA segment of the tube expands significantly, with its OD increasing from 4.0 to 5.6 mm and its ID from 2.1 to 3.4 mm. This change takes place over about 30 min and it is also reversible, *i.e.*, the original diameter is restored when the pH is lowered.

A variation of the experiment with the DMAA–NIPA–DMAA tube is demonstrated by Movie 1 (ESI†), and stills from this movie are shown in Fig. 4d. Here, rather than immersing the tube in a given solution, we flow water through the lumen of the tube. When hot water (above 32 °C) is flowed through the tube, the movie shows the central NIPA segment constricting and becoming opaque in real time (within 20 s). In contrast, the flanking DMAA segments of the tube remain at their original diameter. Thus, the above tube has the ability to *self-regulate* its diameter over specific sections in response to the properties of

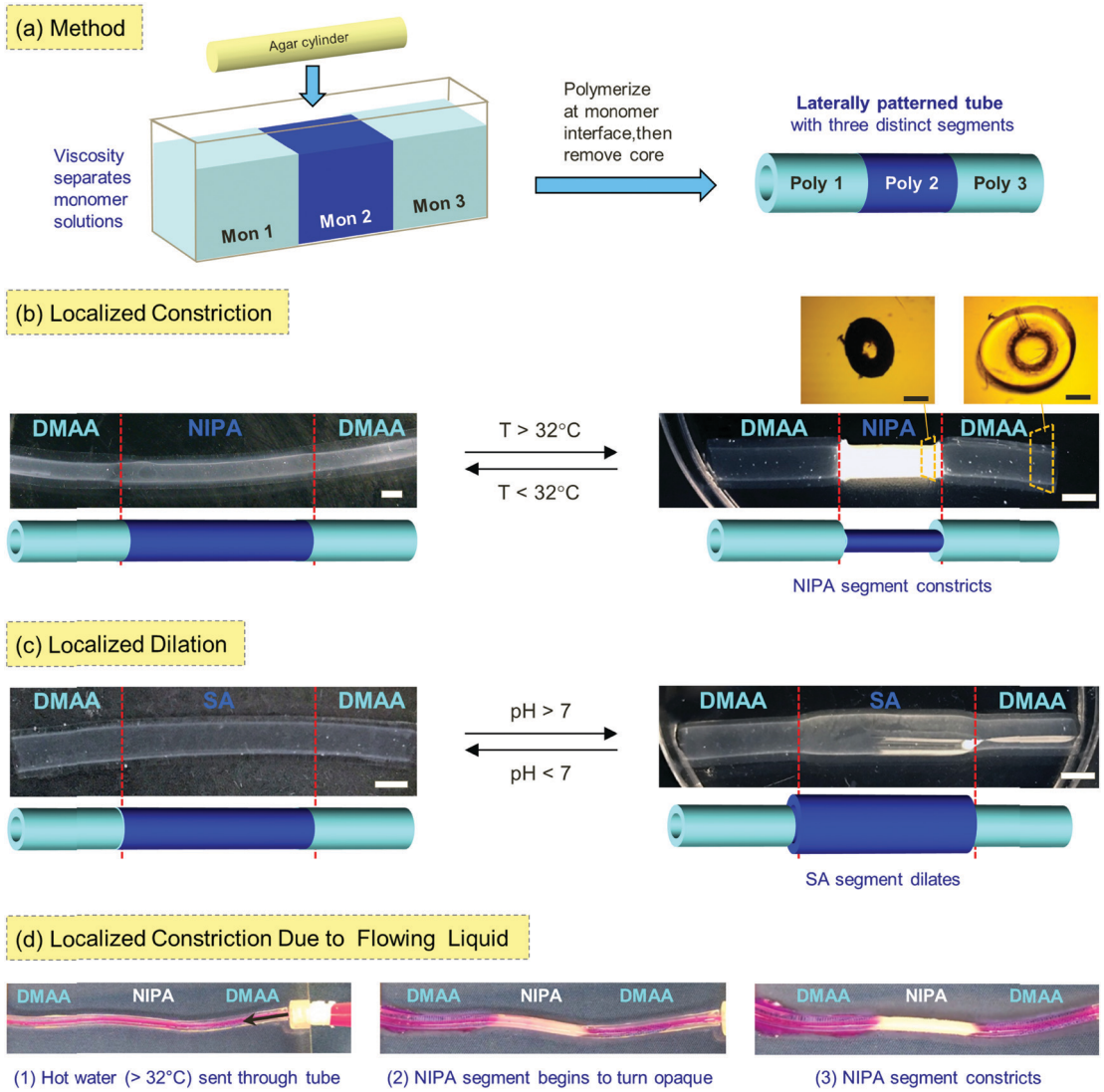

Fig. 4 Synthesis of laterally patterned tubes and examples of the same. (a) The synthesis is achieved by placing an initiator-loaded agar cylinder in a rectangular trough containing two or more viscous monomer solutions, as shown. Upon polymerization, a tube with distinct lateral segments is obtained. (b) Example of a tube with three lateral segments corresponding to DMAA–NIPA–DMAA. When placed in water at a temperature T above 32 °C, the middle NIPA segment of the tube constricts. The inner diameter (see inset micrographs) of the NIPA segment is reduced from 2.1 to 1.0 mm whereas the inner diameter of the DMAA segments remains at 2.1 mm. (c) Example of a tube with three lateral segments corresponding to DMAA–SA–DMAA. At pH > 7, the SA segment becomes ionized and swells; thereby, this segment of the tube dilates, with its inner diameter increasing from 2.1 to 3.4 mm. (d) Localized constriction in a DMAA–NIPA–DMAA tube, as in (b), can be induced by flowing hot water (T > 32 °C) through the tube. This is demonstrated by Movie 1 (ESI†), and stills from the movie are shown in the three panels. The water flowing through the tube is dyed pink. Panels (2) and (3) show the NIPA segment turning opaque and constricting. Scale bars are 5 mm in the photos in (b) and (c), and 2 mm in the inset photos in (b).

the fluid flowing through it. Such a response is akin to blood vessels that constrict or dilate in response to chemical cues. To our knowledge, such behavior has not been demonstrated before in the case of synthetic polymer tubes.

Tubes with longitudinal patterns

Next, we describe the synthesis of tubes with longitudinal patterns – where the top and bottom halves of the tube are made from distinct polymers (Fig. 5a). Such a tube is akin to a Janus or two-faced material. To make these tubes, we again use viscosity to separate two monomer solutions. We pour one solution to fill a rectangular trough half-way to the top. An agar cylinder loaded with initiator is then positioned such that its bottom half is in contact with this solution. Then, we pour a second monomer solution above the first; thereby, the two halves of the cylinder are in contact with different monomers. Again, due to their high viscosity, the adjacent solutions do not mix. After polymerization, the agar core is melted away to yield the patterned tube with distinct top and bottom halves (Fig. 5a).

A resulting patterned tube with a top half of DMAA and a bottom half of NIPA (both layers crosslinked with BIS) is shown in Fig. 5b. At room temperature, this tube is a long, extended structure that seems uniform, *i.e.*, it is not evident from the photo that it is actually anisotropic and Janus-like. The anisotropy does

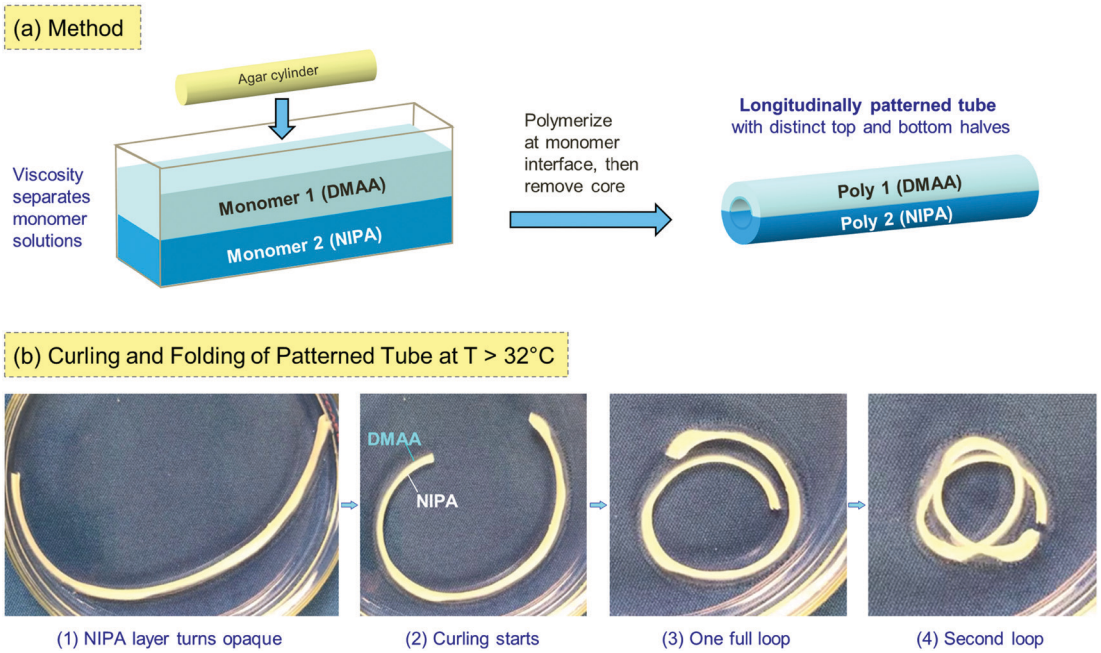

Fig. 5 Synthesis of longitudinally patterned (''Janus'') tubes and an example of the same. (a) The synthesis is achieved by placing an initiator-loaded agar cylinder in a rectangular trough at the interface between two viscous monomer solutions, added one on top of the other. Upon polymerization, a tube with distinct longitudinal segments (top and bottom) is obtained. (b) Example of a tube having a top half of DMAA and a bottom half of NIPA. When heated above 32 °C, the NIPA half turns opaque and shrinks. The mismatch in swelling-degree between the DMAA and NIPA segments causes the tube to curl and coil, as shown by Movie 2 (ESI†), and stills from this movie are provided in the four panels here.

manifest in the response of the tube when placed in water at a temperature above the LCST of NIPA (32 °C). This is revealed by Movie 2 (ESI†) and stills from this movie are provided in Fig. 5b. First, the lower half of the tube becomes opaque due to the phase-separation of NIPA,[27,29] and this makes the Janus-like structure of the tube evident. Thereafter, the tube starts bending and curling,

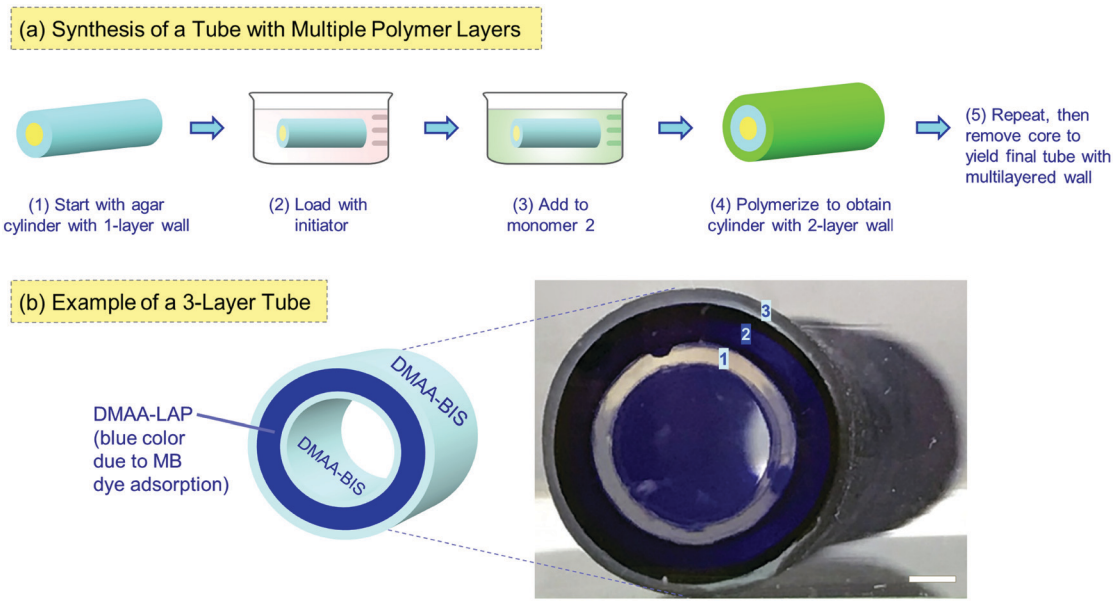

Fig. 6 Synthesis of multilayer tubes and an example of the same. (a) Schematic of the method used. An agar cylinder with a 1-layer wall (made as described in Fig. 1) is used as a new template for the synthesis of the next outside layer (1). The cylinder is soaked in initiator (2) and then placed in a monomer-2 solution (3), yielding a layer of polymer 2 outside the first layer (4). This process is repeated to form additional layers; then, the core is removed to yield the final tube (5). (b) Example of a 3-layer tube is shown by the photo and the schematic. The layers are all gels of DMAA, but differ in the crosslinker used: layers 1 and 3 have BIS as crosslinker while layer 2 has LAP as crosslinker. The differences between the layers are highlighted by selective staining: only layer 2 that has the LAP strongly binds MB dye, which gives it a dark blue color. The scale bar in the photo is 2 mm.

and within about 30 s, it is transformed into a coil with one full turn. The coil continues to curl and adds a second tighter turn over the next 40 s. On the whole, the tube undergoes a heat-induced transformation in shape from a straight segment to a curled loop. This shape change is due to the shrinking of the NIPA half compared to the DMAA half. The swelling mismatch between the top and bottom halves of the tube creates internal stresses.[2,5] To relieve these stresses, the tube curls with the swollen (DMAA) portion outside and the shrunken (NIPA) portion inside. Similar heat-induced curling has been reported to occur for flat sheets,[2,3] or solid cylinders[30] formed by sandwiching gels of DMAA (or other non-responsive polymers) and NIPA.

Multilayer tubes

Our technique can be extended to synthesize multilayer tubes, as outlined in Fig. 6a. For this, a cylinder with one polymer layer (with agar intact in the core) is incubated in the APS initiator solution for 15 min (Steps 1, 2), thereby re-activating the structure for another polymerization.[8] The initiator-loaded cylinder is then transferred into a second monomer solution (Step 3). The initiator diffuses out and induces 'inside-out' growth of a second polymer layer distinct from the first layer and on its outside (Step 4).[8] This process can be further repeated to form additional layers, after which the agar core is removed to yield the final tube. Both the composition and thickness of each layer of the tube can be controlled.

As an example, a 3-layer tube is shown in Fig. 6b, with the layers being: (1) DMAA–BIS, (2) DMAA–LAP and (3) DMAA–BIS. Here, the three layers are based on the same monomer, *i.e.*, DMAA, but different crosslinkers. The thicknesses of the layers are different (500 μm for layer 1, 900 μm for layer 2, 500 μm for layer 3), which is achieved by varying the incubation time of the template in the three successive monomer solutions. To show the differences between the three layers, the entire tube is exposed to MB dye for 24 h and then washed. Only the LAP layer holds onto the MB dye whereas the dye gets washed off from the other layers. Thus, in Fig. 6b, the inner and outer layers are colorless whereas the middle layer is dark blue. This multilayer

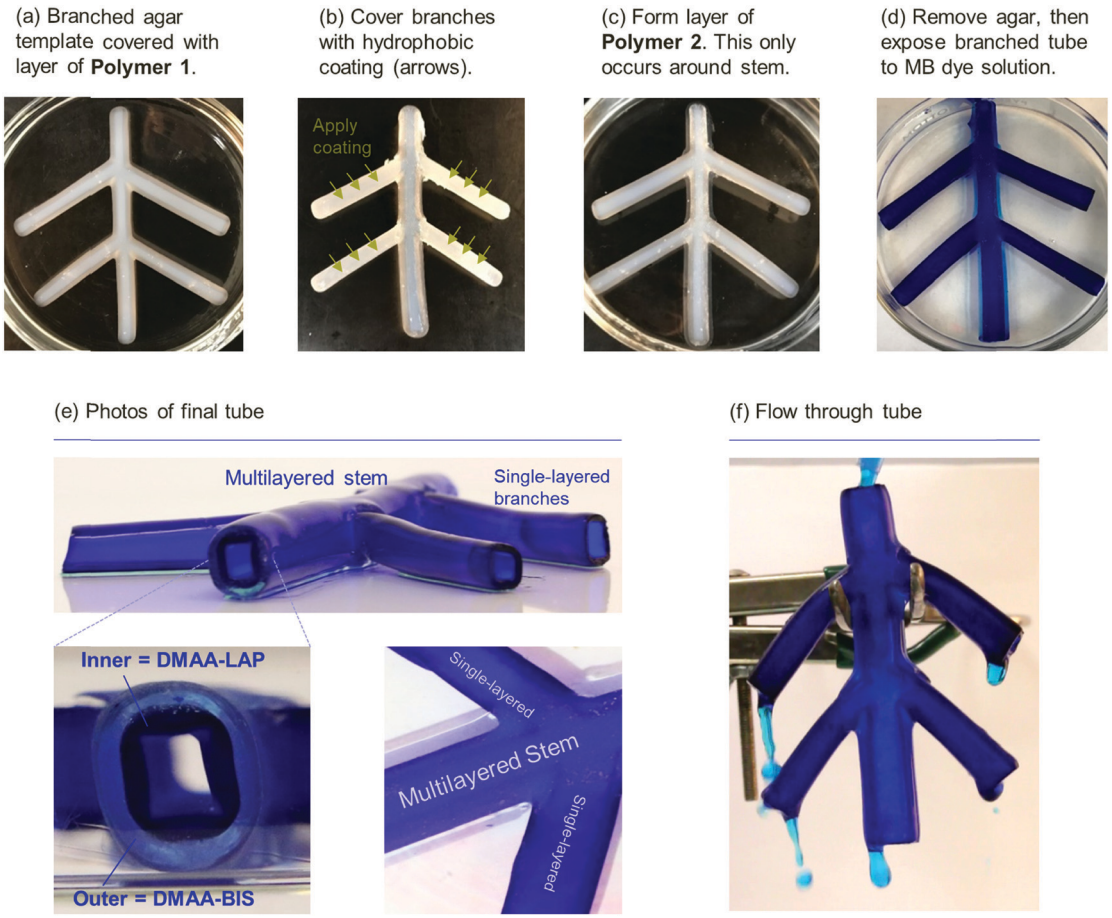

Fig. 7 Synthesis of a branched tube with a multilayered stem and single-layered side branches. (a) A mold of a branched cylinder is first created in agar, and a layer of Polymer 1 (DMAA–LAP) is formed over it using the method shown in Fig. 1. (b) The side branches alone are covered with a hydrophobic coating (c) The partly-coated mold is used as the template to form Polymer 2 (DMAA–BIS); note that this layer forms only around the uncoated stem. (d) The agar core is removed to yield a branched hollow tube, and thereafter the tube is exposed to MB dye, then washed. (e) The final result is a tube with a central stem that has two layers (inner = DMAA–LAP, outer = DMAA–BIS) and branches that have only one layer (DMAA–LAP). The photos clearly reveal the two layers in the stem and the transition from the stem to the branches. (f) Movie 3 (ESI†) depicts the flow of water (dyed light blue) through the tube. A still from the movie is shown here. Note that water flows through both the central stem and all the side branches.

tube is also flexible and robust, much like the tube shown in Fig. 2. Note that our technique allows the stiffness (elastic modulus) and stretchability (degree of elongation) of each layer to be independently controlled, based on its monomer and crosslinker content.

One feature of blood vessels is that they are branched networks with only some segments being multilayered (Fig. S1, ESI†). The narrowest vessels (capillaries) have single-layered walls while the central stem is wider and has a multilayered wall.[10,11] We have attempted to synthesize branched tubes with the above architecture, as shown in Fig. 7. For this, we start with an agar mold that has branches emanating from a central segment. A layer of DMAA–LAP is formed everywhere around this mold (Fig. 7a). Then, the branches alone are coated with a commercially available hydrophobic coating. When the coated structure (Fig. 7b) is loaded with fresh initiator and placed in a second monomer solution, the second polymer layer (DMAA–BIS) forms *only around the central stem* and not the coated branches (Fig. 7c). Next, the agar core is removed and the branched tube is dyed with MB. This reveals the multilayered structure of the central stem (blue inner layer, clear outer layer), which is distinct from the single-layered branches of the tube (Fig. 7d and e). Fig. 7f is a still from Movie 3 (ESI†), which shows liquid flowing through both the central stem as well as all the branches of the above tube. This example illustrates the versatility of our synthesis method since it can be used to create complex 3-D tubular structures while still allowing control of polymer–gel composition, thickness, and elasticity over each region of the structure.

Post-synthesis modification of multilayer tubes

Our multilayer tubes can be modified further after synthesis. One reason to do this could be to introduce chemical functionalities into specific layers or to specific regions of a tube. As an example, we describe how to decorate a specific layer of a tube with fluorescent markers (Fig. 8). We start with a two-layer DMAA–SA tube. The outer layer is DMAA while the inner layer is a copolymer of SA and DMAA in a 10 : 90 molar ratio (both layers are crosslinked with BIS). This tube is added to a solution containing the coupling agents EDC and NHS (see Experimental section), and thereafter reacted with a primary amine, which in this case is fluorescein–amine (Fluor–NH_2). The reaction scheme is intended to conjugate the amine to some of the carboxylate groups present on SA (Fig. 8a). Following reaction and washing, a photo of the modified tube is shown in Fig. 8a, and fluorescence micrographs of the tube-cross-section are provided in Fig. 8b. As expected, we find that the Fluor–NH_2 is indeed conjugated to the inner SA layer, due to which this layer shows a green fluorescence. There is no chemical alteration in the outer DMAA layer, indicating that the reaction is selective to one layer. Similar synthetic modifications could enable new properties to be imparted to our tubes. For example, the inner wall of a tube could be decorated with sensing moieties that could sense analytes flowing through the tube, or with

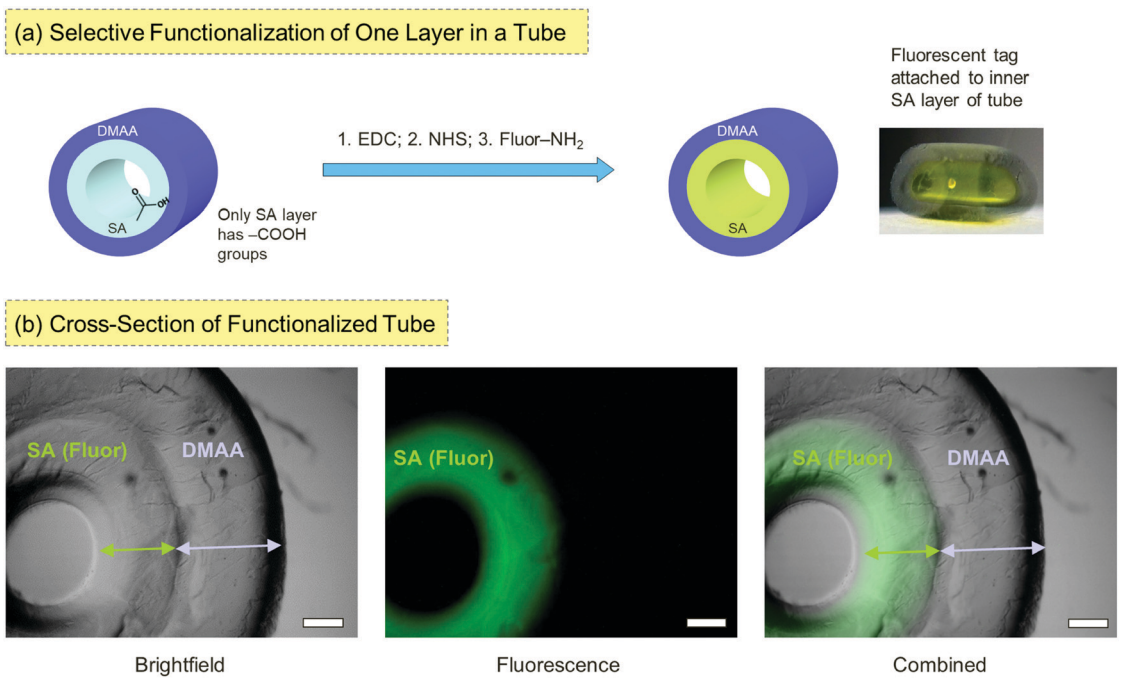

Fig. 8 Selective chemical modification of a specific layer in a multilayer tube. (a) Schematic of the synthesis. The tube has an outer layer of DMAA and an inner layer of SA, with the latter having carboxylate groups. It is reacted with EDC and NHS, and then with a primary amine. The amine chosen here is fluoresceinamine (Fluor–NH_2). A photo of the tube after reaction shows that the inner layer is indeed tagged with fluorescent molecules, due to which this layer has a yellow-green color. (b). The tube-cross-section is further examined by fluorescence microscopy, and the images show that the inner layer has a bright green fluorescence whereas the outer layer does not. Scale bars in the images are 1 mm.

biological molecules (*e.g.* growth factors) to enable the use of these tubes in tissue engineering.

Conclusions

We have presented a new technique for the synthesis of polymer tubes in the laboratory without the need for complex infrastructure like 3-D printers. Our technique provides precise control over lumen diameter, wall thickness, numbers of layers in the wall, and the chemistry of individual layers. Tubes synthesized by this method are mechanically robust and their mechanical properties can be easily tuned by varying the layer composition. A key advance over existing methods is that tubes can be patterned with different polymers either in the lateral or longitudinal directions. Patterned tubes based on stimuli-responsive polymers exhibit the ability to spontaneously change their lumen diameter in response to stimuli, or to convert from a straight to a curled shape. Tubes can also be post-modified to attach chemical moieties selectively to a given layer of a multilayer wall. It is worth noting that we have not explored the limits of our approach in terms of the finest resolution that can be achieved, either in the thickness of a given layer in the tube wall or in the sizes of lateral or longitudinal patterns. For example, very thin walls ($\sim$ in the tens of microns) can be realized by using a low polymerization time ($\sim$1 min) and a low initiator concentration in the core template.[8]

Tubes synthesized using this method could have a variety of applications. They could be used as stand-alone structures as reactors to conduct aqueous chemical reactions. They could be used as channels or conduits in a soft microfluidic device, or embedded within gels. The most obvious applications would be in biomedical or tissue engineering, where such tubes could serve as mimics of blood vessels (vascular grafts) or other tubular tissues.[12,13,15] In this regard, it is important to note that the tube walls can be made using a variety of polymers that have already been used in biomedical studies. These include gels of acrylate, methacrylate, or acrylamide monomers such as polyethylene glycol diacrylate, which are widely employed as scaffolds for cell culture.[5,6] In addition, methacrylated derivatives of biopolymers like gelatin or alginate,[13,14] which are popular in the 3D-printing of biomaterials, could also be used to create the tube walls. Importantly, since the tube walls are synthesized under mild, aqueous conditions, the encapsulation of cells (as well as biomolecules such as enzymes and DNA) can be done in a single step during synthesis. In the case of multilayer tubes, different cell types can be encapsulated and grown in each of the layers. Thus, our study suggests the possibility of creating more realistic mimics of the tubes in our body, in terms of both the structure of the tube walls as well as the ability of the tubes to respond to solutes in the liquid flowing through the tubes.

Experimental section

Materials

The following were purchased from Sigma-Aldrich: the initiator ammonium persulfate (APS); the accelerant *N*,*N*,*N*′,*N*′-tetramethylethylenediamine (TEMED); the monomers sodium acrylate (SA), *N*,*N*′-dimethylacrylamide (DMAA), and *N*-isopropylacrylamide (NIPA); the crosslinker *N*,*N*′-methylenebis(acrylamide) (BIS); the cationic dye methylene blue (MB); xanthan gum (XG); fluoresceinamine (F–NH_2); and *N*-hydroxysuccinimide (NHS). The coupling agent 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide (EDC) was purchased from Carbosynth. Agar was purchased from Living Jin (the product is termed agar–agar and is extracted from seaweeds). The nanoclay LAPONITE® XLG (LAP) was obtained as a gift from Southern Clay Products. Deionized (DI) water from a Millipore system was used for all experiments. Rust-Oleum Never-Wet spray was purchased from Home Depot.

Synthesis of agar template cylinders

Agar powder was dissolved in $\sim$90 °C DI water at a concentration of 5%. This hot solution was then inserted into a length of Tygon® tubing (from Cole Parmer) of the desired inner diameter. After $\sim$30 min at room temperature, the agar solidifies. Water was then injected into the tube end to force the agar cylinder out of the tubing. The cylinder was then cut to the desired length using a razor blade. Tubes were stored in DI water at 4 °C until use.

Basic synthesis of polymer tubes

First, the agar template cylinder was incubated in a 15 mg mL^{-1} solution of APS. After 15 min, the cylinder was removed, blotted with a Kimwipe®, and placed in a monomer solution contained in a rectangular trough or a deep Petri dish. A typical monomer solution had 10% monomer, 0.34% BIS (crosslinker), 0.5% XG, and 15 mg mL^{-1} TEMED. The XG was added to increase the viscosity of the solution; note that the template stayed suspended in the middle of the viscous solution in the container, allowing polymerization to occur on all sides of the cylinder.[8] Instead of BIS, LAP particles were also used as cross-linkers,[23,24] and in that case, the composition was: 10% monomer, 0.05% BIS, 3% LAP and 15 mg mL^{-1} TEMED. The XG was not used with the LAP because the LAP particles themselves aggregate and make the solution viscous. Following synthesis, the cylinder was removed from solution and washed several times with water. A razor blade was used to cut the caps off the cylinder, thus exposing the agar. Thereafter, the whole structure was placed in water at $\sim$90 °C to melt the agar and thereby remove the core. This finally yielded the desired tube. In some cases, the agar core could be pushed out without heat simply by applying mechanical force on the core at one end. To ensure full removal of the agar core, injection of water through the lumen of the tube using a syringe was found to help.

Staining of tubes with LAP-containing layers was done using a 10 μM solution of methylene blue (MB).[23,24] The tube was soaked in the MB solution for a given period of time and then removed and washed several times with water. Strong adsorption of MB onto LAP particles gave the LAP-containing layers a permanent blue color, as shown in Fig. S3 (ESI†). In contrast, any dye in the other layers was removed during the washing steps. Also, the color of the LAP layers could be changed from a lighter shade of blue for a short (30 min) soak to a dark blue for a soak of 9 h or more.

Synthesis of multilayer, patterned, and branched tubes

Variations of the above method have been discussed further in the Results and discussion section. Additional details are noted here. To synthesize multilayer tubes, following synthesis of the first layer around an agar core, the cylinder was washed and reloaded with initiator. The layer synthesis was then carried out with a different monomer solution. After the desired number of layers was synthesized, the agar core was finally removed by heat.

For laterally patterned tubes, a rectangular trough of dimensions 7.5 × 2.5 × 2.5 cm was built by gluing glass slides together using epoxy. To create the three-segmented tube in Fig. 4, two further glass slides were placed vertically in the trough at equidistant points and used as 'separators'. This divided the trough into three chambers. Different monomer solutions were poured in the three chambers. Each solution had 10% of the monomer and was rendered viscous by adding either 3% LAP or 0.5% XG. Thereafter, the separators were removed. Due to their viscosity, the laterally segregated monomer solutions did not mix significantly.[24] Next, an agar cylinder (typical diameter 2–5 mm, typical length ~5 cm) loaded with initiator was placed perpendicularly across the three chambers. Polymerization was conducted for 30 min, following which the agar core was removed by heat.

For longitudinally patterned tubes, the same trough as above was used. Two solutions of viscous monomers were then prepared; the first was poured halfway to the top of the trough. An agar cylinder, similar to the one above, was then carefully placed in such a way that its bottom half was in contact with this solution. Next, the second viscous monomer solution was poured over the first; thereby, the two halves of the cylinder were in contact with different monomers. Polymerization was again conducted for 30 min, following which the agar core was removed by heat.

To create branched tubes, an agar mold was first made in the desired shape. For this, an agar gel was first made in a Petri dish, and this gel was cut into the desired shape using a razor blade. The synthesis of a first polymer layer around the agar template was done in the usual way, as described above. Next, the branches alone were coated with the Rust-Oleum Never-Wet spray. This results in a hydrophobic translucent coating around the branches. The coated structure was loaded with initiator and placed in a second monomer solution, resulting in a second layer only around the uncoated regions. Finally, the agar core was removed by heat. The hydrophobic coating is a discrete, flaky layer and this can also be peeled away, if necessary, using forceps.

Fluorescent modification of polymer tubes

A two-layer tube was employed with an inner layer being a copolymer of SA and DMAA (10 : 90 ratio by weight of the total monomer) while the outer layer was solely DMAA. The tube was placed in water at pH 4.5 and to this EDC and NHS were added at concentrations that were 1.5× the molar equivalent of the SA in the tube (each SA monomer has a carboxylate group, which is the one that reacts with the EDC). After 30 min of incubation, a solution of 0.01 g mL^{-1} of Fluor–NH_2 in methanol was added dropwise to the above tube while stirring. The amount of Fluor–NH_2 added was half the molar equivalent of the SA. The sample was then covered with aluminum foil to prevent photobleaching and maintained at 65 °C under moderate magnetic stirring for 24 h to allow the reaction to proceed. The tube was then washed once with a 50/50 methanol/water solution and three more times with water adjusted to a pH of 4.5.

Optical microscopy

All microscope images were taken using a Zeiss Axiovert 135 TV inverted microscope. Images were typically taken using a 2.5× objective. A microruler and the image analysis software ImageJ were used to determine tube dimensions from collected images. To visualize the green fluorescence from F–NH_2, which is green, images were collected using a band pass excitation filter (450–490 nm) and a band pass emission filter (515–565 nm). The images were then subsequently combined using ImageJ.

Tensile testing

Tensile tests were conducted on a tube with a NIPA wall (3 mm outer diameter, 0.9 mm wall thickness, and 5 cm length) using an Instron Model 5565 instrument. The tube was gripped by the jaws of the Instron and the sample was elongated at a rate of 10 mm min^{-1}. The force recorded during this process was converted to tensile stress and plotted against the tensile strain.

Conflicts of interest

There are no conflicts of interest to declare.

Acknowledgements

We acknowledge Matthew Bohensky, Felix Yuwono, Ankit Gargava, Kerry DeMella, and Leah Borden for their contributions to some of the experiments described in this paper. We also acknowledge Prof. John Fisher for allowing us the use of the Instron instrument in his lab.

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Electronic Supplementary Material (ESI) for Soft Matter.

Supporting Information for:

Multilayer Tubes that Constrict, Dilate, and Curl in Response to Stimuli

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Contents

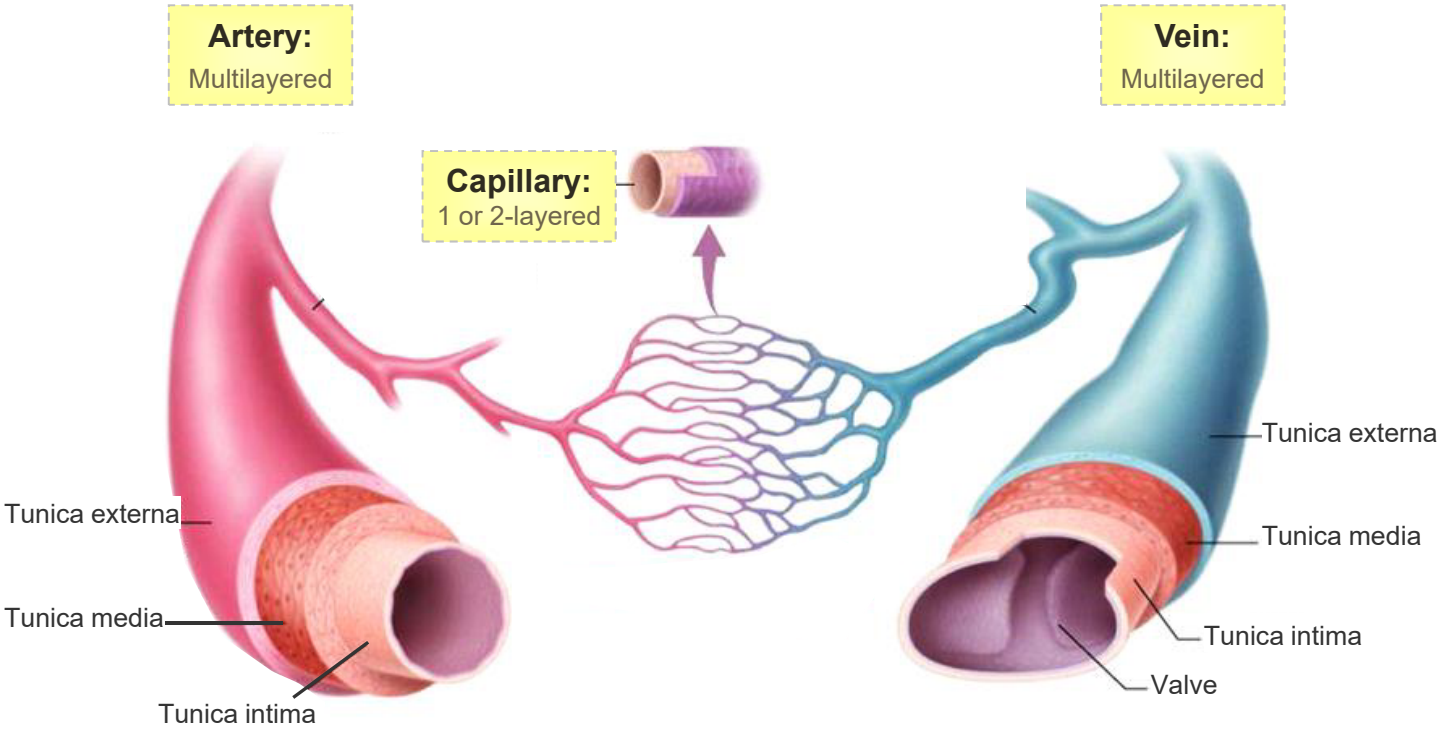

Figure S1. Anatomy of blood vessels. The veins and arteries have the largest inner diameters (up to 30 mm) and these taper off into venules and arterioles, which then further taper off into capillaries (with diameters as small as 8 μm). The capillaries have thin walls with one or two layers. The veins and arteries have thick walls composed of multiple concentric layers, including an innermost layer (*tunica intima*) of endothelial cells, a middle layer (*tunica media*) of smooth muscle tissue, and an outer layer (*tunica externa*) of connective tissue.

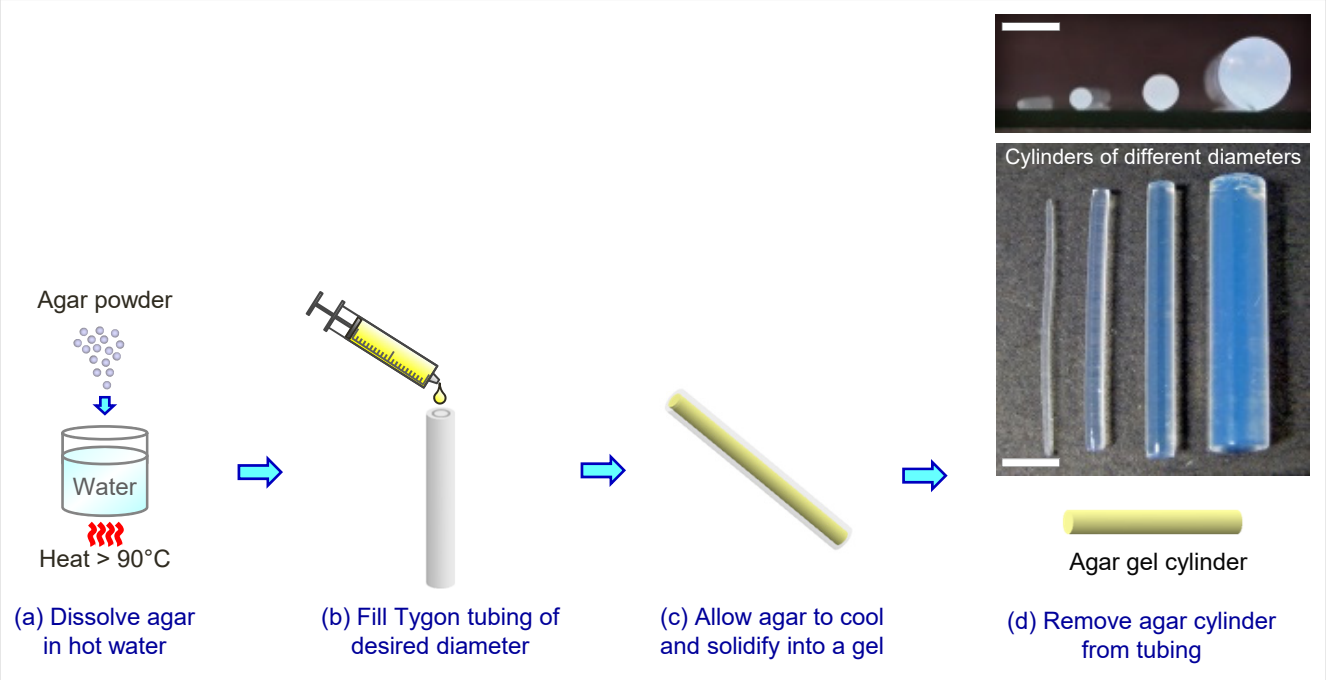

Figure S2. Preparation of agar gel cylinders. (a) Agar is dissolved in hot water. (b) The hot agar solution is poured into a piece of Tygon tubing of desired length and diameter. (c) Upon cooling, an agar gel is formed in the tube. (d) The cylindrical gel is removed from the tube by injecting water into one end of the tube. This cylinder is used as the template for the synthesis shown in Figure 1. The top panels in (d) show agar cylinders of various diameters in top and front views, and the scale bars in the images are 4 mm.

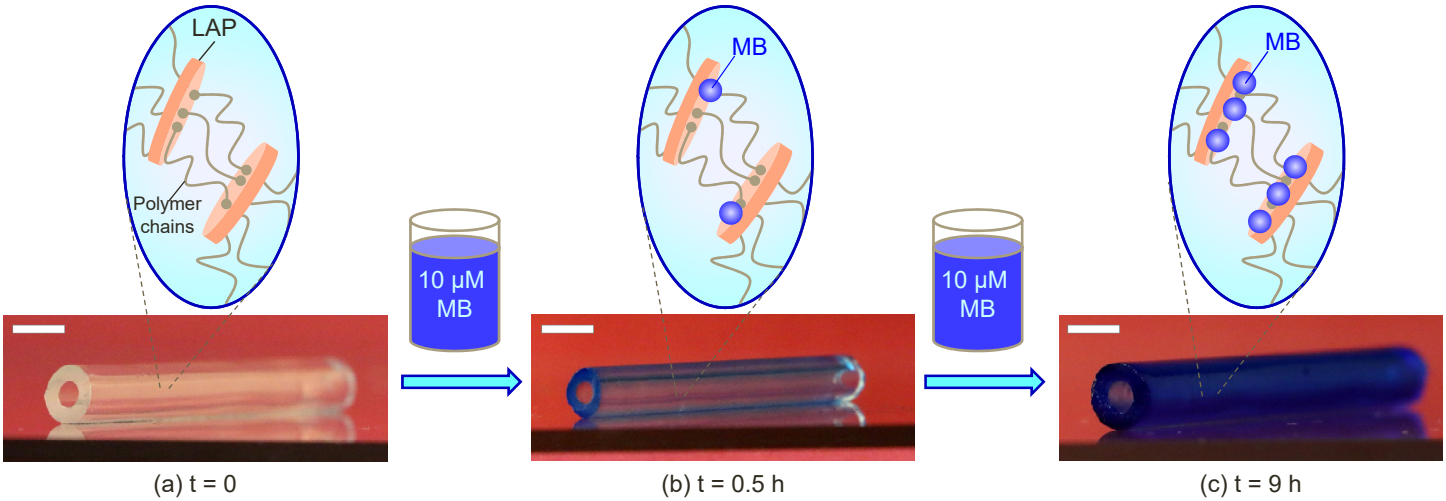

Figure S3. Tube with a polymer layer crosslinked by laponite (LAP) nanoparticles and staining of this layer. LAP particles serve as crosslinkers for growing polymer chains, leading to a polymer network, as shown by the schematics. A tube with a layer of DMAA-LAP is transparent (a). When placed in a 10 μM solution of the cationic dye, methylene blue (MB) for 30 min, the tube wall takes on a light-blue color (b). Incubation in the same MB solution for 9 h gives it a dark blue color (c). The color is due to irreversible adsorption of the MB on the anionic faces of the LAP particles, as shown by the schematics. Scale bars in the images are 4 mm.

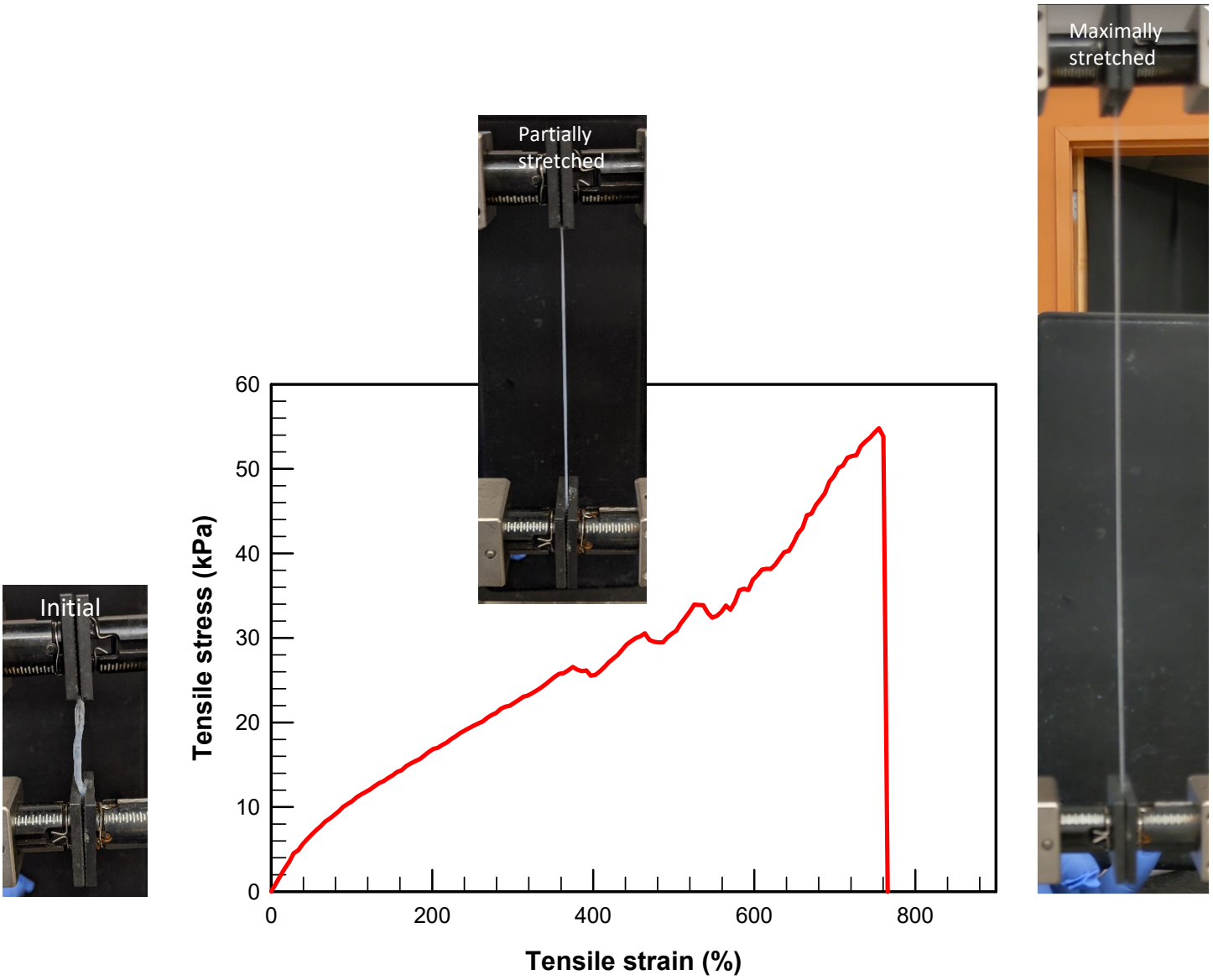

Figure S4. Mechanical properties of tubes under tension. A plot of tensile stress vs. strain is shown for a tube with a single layer of N-isopropylacrylamide (NIPA, 10%) crosslinked with laponite (LAP, 3%) particles. Photos of the tube at diffferent extents of elongation are also shown. The tube can be stretched up to a strain of 750% before it ruptures. From the initial linear portion of the stress-strain curve, the Young's modulus is calculated to be 20 kPa.

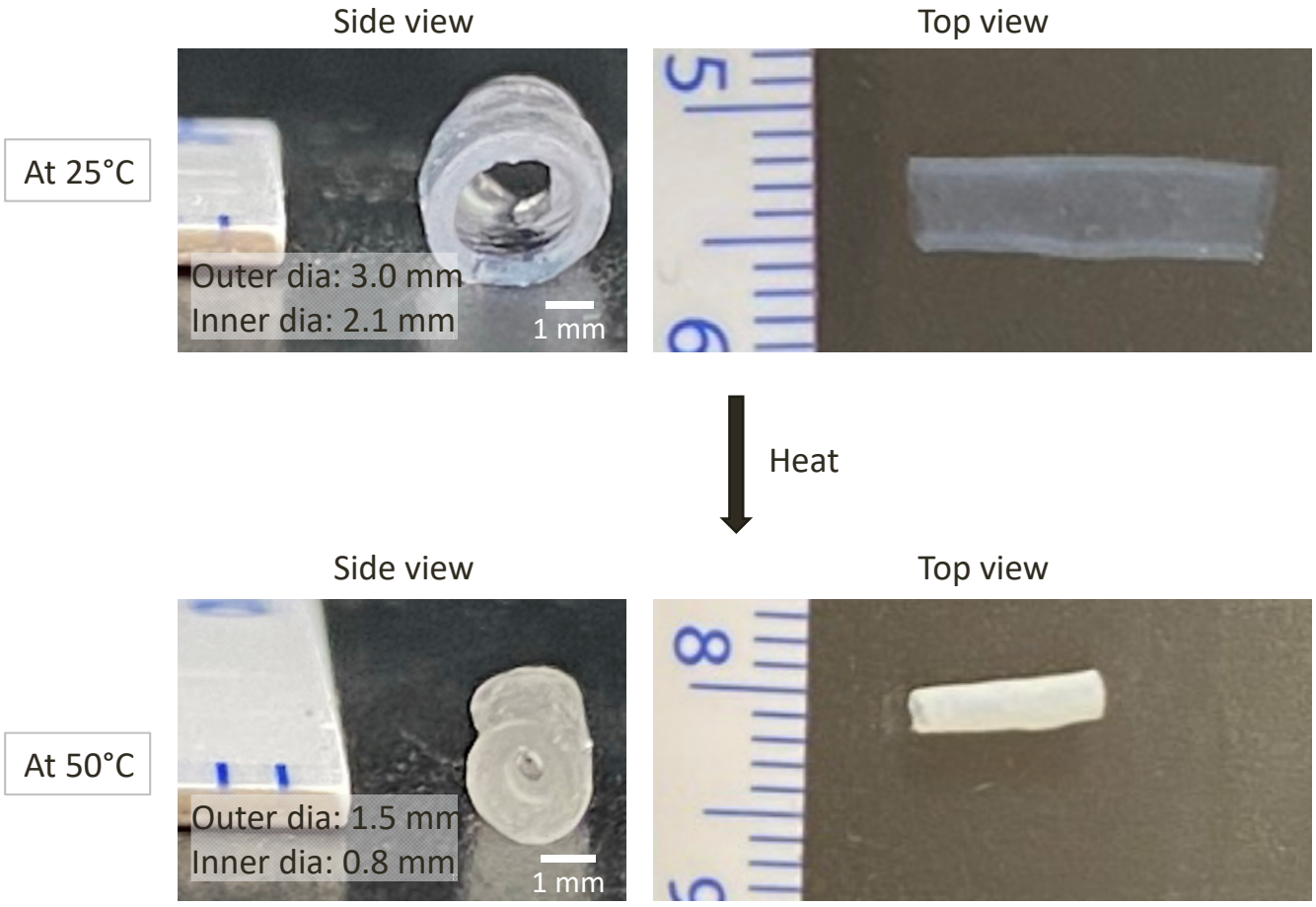

Figure S5. Constriction of a NIPA tube upon heating. The tube with a NIPA wall is shown in two views (side and top) at 25°C and 50°C. At the low temperature, the tube is nearly clear and has an inner diameter of 2.1 mm and an outer diameter of 3.0 mm. When heated above the LCST of NIPA, the tube constricts and becomes opaque. The outer diameter is reduced to 1.5 mm and the inner diameter to 0.8 mm. Similar data are shown for a patterned tube of DMAA-NIPA-DMAA in Figure 4.