ACS APPLIED MATERIALS & INTERFACES

Research Article

Cite This: ACS Appl. Mater. Interfaces 2019, 11, 37103−37111

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Rapid Electroformation of Biopolymer Gels in Prescribed Shapes and Patterns: A Simpler Alternative to 3-D Printing

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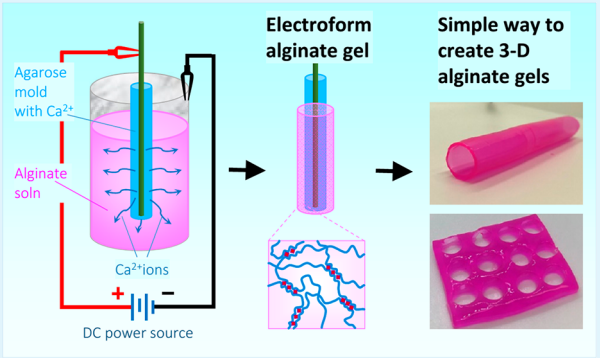
Supporting Information

ABSTRACT: We demonstrate the use of *electric fields* to rapidly form gels of the biopolymer alginate (Alg) in specific three-dimensional (3-D) shapes and patterns. In our approach, we start with a gel of the biopolymer agarose, which is thermoresponsive and hence can be molded into a specific shape. The agarose mold is then loaded with Ca^{2+} cations and placed in a beaker containing an Alg solution. The inner surface of the beaker is surrounded by aluminum foil (cathode), and a copper wire (anode) is stuck in the agarose mold. These are connected to a direct current (DC) power source, and when a potential of ∼10 V is applied, an Alg gel is formed in a shape that replicates the mold. Gelation occurs because the Ca^{2+} ions electrophoretically migrate away from the mold, whereupon they cross-link the Alg chains adjacent to the mold. At low Ca^{2+} (0.01 wt %), the Alg gel layer grows outward from the mold surface at a steady rate of about 0.8 mm/min, and the gel stops growing when the field is switched off. After a gel of desired thickness is formed, the agarose mold can be melted away to leave behind an Alg gel in a precise shape. Alg gels formed in this manner are transparent and robust. This process is particularly convenient to form Alg gels in the form of hollow tubes, including tubes with multiple concentric layers, each with a different payload. The technique is safe for encapsulation of biological species within a given Alg layer. We also create Alg gels in specific patterns by directing gel growth around selected regions. Overall, our technique enables lab-scale manufacturing of alginate gels in 3-D without the need for an expensive 3-D printer.



KEYWORDS: *alginate, agarose, electrogelation, electropatterning, electrophoresis*

INTRODUCTION

Polymer hydrogels are a quintessential class of soft materials.[1−3] A hydrogel is a sample-spanning, three-dimensional (3-D) network of polymer chains, with the aqueous solvent trapped in the network. Hydrogels can be broadly classified into two categories: chemical and physical gels. Chemical gels are formed by the polymerization of a solution containing monomers and cross-linkers, with the resulting polymer chains being connected by covalent cross-links. Physical gels are formed by inducing physical cross-links between long polymer chains. An important example of a physical gel is that of alginate (Alg).[4−8] In this case, a solution of sodium alginate, an anionic polysaccharide, is combined with a salt of a divalent cation like calcium (Ca^{2+}). The Ca^{2+} cations cross-link the alginate chains through ionic bonds, thereby leading to a physical gel of alginate (denoted as Alg in the rest of the paper). Alg gels are extensively used in biomedical applications due to their nontoxic, biocompatible nature and due to the mildness of the gelation process.[9,10] In particular, Alg gels are used to encapsulate a range of biological cells. For this, the cells of interest are mixed initially with the Alg solution, and this mixture is then combined with the cross-linking solution (containing Ca^{2+} ions) to form a gel with embedded cells.[9,10]

Recently, researchers have sought other ways to manufacture gels, both physical and chemical. One motivation for this research has been the advent of methods like additive manufacturing (3-D printing), which promise to enable the synthesis of materials with precise shapes and properties. While 3-D printing is routinely implemented with thermoplastic polymers, its extension to biomedically relevant soft structures will require its adaptation to hydrogels, such as those of Alg.[11−13] For example, to manufacture a tissue-like material, it would be useful to create multilayered Alg gels, with each layer having a precise thickness and containing a specific type of cells. Also, such materials will need to be created in accurate shapes and patterns. For such precision-manufacturing, the current Alg gelation process of combining an initial Alg solution with a solution of a cross-linker is not well-suited, and, indeed, current attempts at 3-D printing Alg gels are cumbersome.[13−15] There is thus a need for alternate

Received: July 17, 2019
Accepted: September 17, 2019
Published: September 30, 2019

DOI: 10.1021/acsami.9b12575
ACS Appl. Mater. Interfaces 2019, 11, 37103−37111

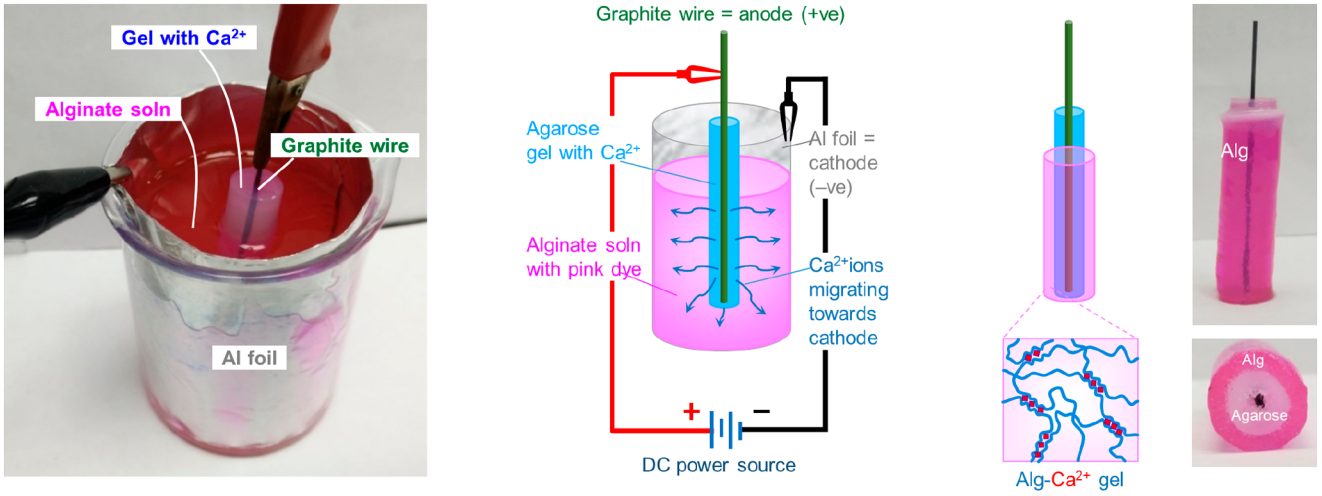


Figure 1. Procedure for electrogelation of alginate (Alg). The photo in (a) and the schematic in (b) show the setup. The key components are an agarose gel containing Ca^{2+} with a graphite wire in it and a beaker covered on its inside with aluminum (Al) foil and filled with a solution of Alg. The Al acts as the cathode and the graphite as the anode. When a DC field is turned on, Ca^{2+} ions migrate out of the mold and encounter Alg chains at the mold surface. Cross-linking of Alg chains by Ca^{2+} leads to an Alg gel around the mold, as shown in (c). Photos show the agarose core surrounded by a shell of the Alg gel, which has a pink-red color due to the inclusion of a dye in the Alg solution.

techniques that allow an Alg gel to be formed upon the flick of a switch (i.e., so that there is a precise "start") and conversely for this formation to cease when the switch is turned off (i.e., to ensure a precise "stop"). Possible ways to achieve start–stop gelation are using external stimuli such as light and electric fields.[16−23] Light-induced physical gelation of Alg has been shown, for example, using chemicals that release Ca^{2+} upon shining ultraviolet (UV) light.[17,18,20] However, these techniques are relatively slow and mostly suited to forming Alg gels as thin films rather than as 3-D materials.

Electric fields are another possibility for start–stop gelation. The question is whether one could switch on gelation of a polymer upon passing a current through a solution and then stop the gelation upon turning off the current. So far, to our knowledge, electrodeposition of thin films on electrodes has been widely shown, as summarized in a recent review by Boulmedais et al.,[24] but not the formation of gels in bulk or in specific geometries. In regard to electrodeposition, systematic studies have been done by Payne et al., first with the cationic biopolymer chitosan[25] and thereafter also with Alg.[26,27] In the case of chitosan, the authors exploited the fact that, when direct current (DC) is passed through water, electrolysis leads to pH gradients close to the electrodes. Near the cathode, the pH becomes high, whereas near the anode, the pH becomes low. Accordingly, chitosan, which has a pK_a around 6.5, deposits on the surface of the cathode (because the polymer chains lose their charge due to the high pH and thus fall out of solution).[25] Similar electrodeposition has been done with Alg at the anode.[28,29] An alternative approach in the case of Alg is to use the pH drop (release of H^+) at the anode to solubilize calcium carbonate ($CaCO_3$) particles suspended in the solution, resulting in the evolution of free Ca^{2+}.[26,27] These cations gel the Alg, which deposits on the anode. Other electrodeposition schemes have been reported using cations like Fe(II),[30] which are further discussed in the review by Boulmedais et al.[24] However, regardless of the approach, Alg electrodeposition is restricted to thin films and that too on specific substrates (electrodes). Moreover, in these schemes, when the field is switched off, gelation does not really stop. For example, if gelation is due to a pH gradient in solution, this gradient will continue to exist for some time after the field is switched off.

In this paper, we describe a new technique to form Alg gels upon applying an electric field. Our technique is rapid and biocompatible and relies on electrophoretic migration rather than electrolysis of water. Importantly, the technique allows transparent and robust Alg gels to be formed in desired shapes and patterns. The geometrical flexibility of our technique is due to the use of a molded agarose gel as an electrode (which also serves as the substrate for Alg gel growth). Agarose is a nonionic, thermoresponsive polysachharide; cooling an agarose sol converts it into a gel.[31,32] We place an agarose mold (containing Ca^{2+}) in an Alg solution and apply electrical bias with a specific polarity. An Alg gel is formed around the mold within minutes. Gelation occurs because Ca^{2+} ions and Alg chains migrate toward each other and come into contact at the surface of the agarose mold. The shape of the Alg gel thus becomes an inverse replica of the mold. *An important point is that the use of the electric field allows gelation to be started and stopped on demand*; in turn, this enables the controlled synthesis of gels in 3-D architectures. Specifically, we show the synthesis of Alg tubes having multiple layers and with nanoparticles (NPs) or biological cells embedded in specific layers. The simplicity and versatility of this technique should make it attractive to researchers, and it could emerge as a viable alternative to 3-D printing for custom-fabrication of hydrogels.

RESULTS AND DISCUSSION

Electrogelation Setup and Procedure. Agarose dissolves in water at temperatures around 80 °C. When the agarose sol is cooled down to room temperature, it transforms into a gel.[32] The sol to gel transition is reversible, i.e., the gel can be liquefied by heating. We first made a cylindrical agarose

DOI: 10.1021/acsami.9b12575
ACS Appl. Mater. Interfaces 2019, 11, 37103−37111

gel containing Ca^{2+}. For this, 2.5 wt % of agarose and 0.1 wt % of $CaCl_2$ were added to deionized (DI) water and dissolved by heating to 80 °C. The hot solution was poured into a glass test tube around a graphite electrode and allowed to cool down to room temperature, whereupon the agarose sets into a molded gel. This mold (5.5 cm long, 1 cm diameter) was then used in the electrogelation setup, as shown in Figure 1a,b. In the setup, the graphite is connected to a DC power supply and serves as the anode (positive electrode). The mold is placed in a beaker that is wrapped on its inside with aluminum (Al) foil, which serves as the cathode (negative electrode). Alg (sodium salt) at a concentration of 1 wt % is poured in the beaker, and here, it is dyed pink-red (using 0.5 mM of acid red 52 dye) for visualization.

We then apply an electric potential (typically ~10 V), whereupon the Ca^{2+} ions in the mold begin migrating toward the cathode, i.e., away from the mold (see the schematic in Figure 1b). Correspondingly, Alg chains in the solution also migrate toward the mold (anode). This electrophoresis causes the Ca^{2+} to contact the Alg at the surface of the mold, resulting in an Alg gel layer. Figure 1c shows a pink-red Alg gel around the agarose mold. A schematic of the Alg gel is provided in Figure 1c: note that the divalent Ca^{2+} cations form "egg-box" junctions between the anionic Alg chains, thereby cross-linking the chains into a network.[4−8] The gel layer around the mold grows over time, but when the electric field is switched off, the growth stops. The Alg gel layer in Figure 1c is formed over 5 min of applying the field. From the cross-sectional view, we infer that the layer is 3 mm thick at this point. Next, as indicated by Figure 2a,b, we place the above agarose−Alg

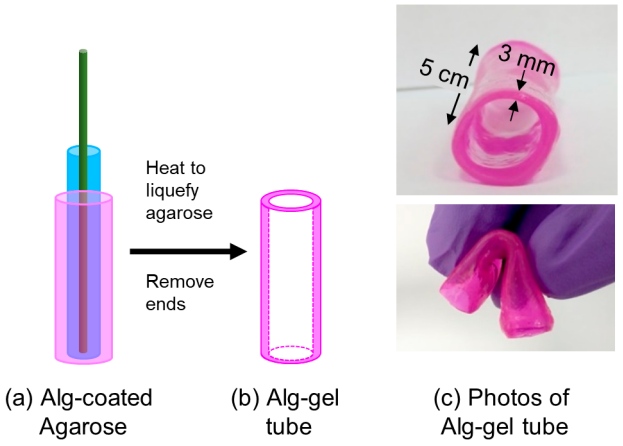


Figure 2. Preparation of a tubular Alg gel. The Alg-coated agarose in (a) (see Figure 1) is heated to 80 °C to liquefy the agarose. This leaves behind a hollow tube of Alg, as shown in (b). Photos in (c) reveal that the Alg tube is strong and flexible.

assembly in a hot water bath at 80 °C, which causes the central agarose mold to be dissolved away, leaving behind a hollow tubular gel of Alg (with the tube wall being 3 mm thick). Figure 2c shows two different photos of this tubular gel. The tube is flexible and bendable, yet strong and robust. If necessary, the tube can be subsequently incubated in a Ca^{2+} solution to increase its mechanical strength. For example, an electroformed tube can be placed in a 5 wt % Ca^{2+} solution for 10−30 min and thereby made more robust.

The above technique is general and can be modified in many ways. First, it is not limited to Ca^{2+} ions. Any multivalent cation that can cross-link Alg (such as Sr^{2+}, Cu^{2+}, Fe^{3+}, and Ho^{3+})[5,7] could be incorporated into the mold and used to electroform the Alg gel. Instead of Alg, other polymers that can be gelled by such cations could also be used (such as pectin[17] or polyacrylic acid[21]). Also, we are not limited to using agarose as the mold. If the mold does not have to be removed, any gel, including chemically cross-linked hydrogels (for example, acrylamides),[2,3] could be used as the Ca^{2+}-containing mold. If the mold needs to be removed at a more moderate temperature, we can replace agarose with gelatin,[9,10] since gelatin gels can be melted around 40 °C. Also, the mold could be degraded at room temperature using enzymes; for instance, a mold made of gelatin could be degraded by enzymes from the collagenase or proteinase families.[33,34] Additionally, molds of any shape and geometry can be used. Examples with disc-like molds and flat-sheet molds are shown below, and in all cases, an Alg gel forms around the mold when the electric field is applied.

Kinetics of Gel Growth. We studied the growth of the Alg gel with time around an agarose mold for different Ca^{2+} concentrations in the mold. For this, the agarose mold was made in the shape of a disc (5.5 mm in diameter, 4 mm in height) and placed in a Petri dish containing 1% Alg solution while being observed (see Experimental Section for details). Figure 3a shows images of the growing Alg gel over 5 min at a potential of 10 V. These images are for the case of 0.01 wt % Ca^{2+} in the agarose mold. The Alg gel can be easily resolved because it is transparent, while the agarose mold is cloudy. The thickness of the Alg gel steadily increases over time (Figure 3b), with the increase being close to linear at ~0.8 mm/min for this low Ca^{2+} concentration. As the Ca^{2+} concentration is increased, a different shape of the plot is seen in Figure 3b: after an initial linear increase, the gel thickness saturates within the 5 min period. Also, Figure 3c shows that, after 5 min, the Alg gel for the case of 1 wt % Ca^{2+} is approximately 1.5 mm thick; for comparison, this layer is about 4 mm thick at the same point of time in Figure 3a when the Ca^{2+} is at 0.01 wt %.

The above result might seem counterintuitive at first. One might expect the thickness to increase with higher availability of Ca^{2+} ions, but the opposite is observed in our experiments. We hypothesize that there is a difference in the nature of Alg gel formation at low and high Ca^{2+}. At high Ca^{2+}, we expect the ions to cross-link the Alg into a dense network.[4−8] This dense network may hinder further migration of Ca^{2+} ions from the interior of the mold and thereby restrict the thickness of the gel. In contrast, the network at lower Ca^{2+} will be less dense, which may allow Ca^{2+} ions to migrate through and thereby give rise to thicker gels. Our results thereby suggest that there are advantages to using relatively low Ca^{2+} (0.01−0.1 wt %) in the mold: i.e., the gels can grow to larger dimensions, as discussed above. A second advantage at these lower Ca^{2+} concentrations is that there is no detectable growth of the gel in the absence of the electrical signal; thus, we have a true "on−off" switch for gel growth. In contrast, when the Ca^{2+} is 1 wt % or higher, a thin gel layer forms slowly around the mold even in the absence of the electric field simply by diffusion. We have measured the rate of gel growth by diffusion at 1 wt % Ca^{2+} (same geometry as in Figure 3) and found the rate to be 17 μm/min, which is about 50 times slower than the rate of gel growth by the electric field. A rheological comparison of gels formed by the field and by diffusion is provided below.

We also observed that when the concentration of Ca^{2+} is low (less than 0.5 wt %), the agarose mold (made with 2.5 wt %

DOI: 10.1021/acsami.9b12575
ACS Appl. Mater. Interfaces 2019, 11, 37103−37111

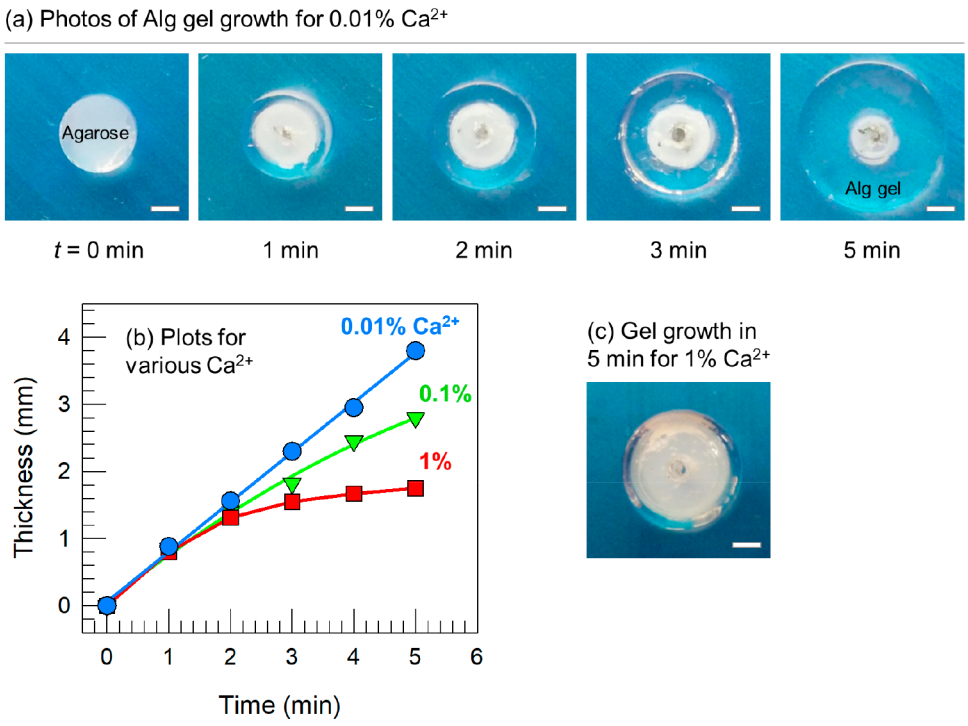


Figure 3. Growth kinetics of electroformed Alg gel. A 2.5% agarose disc with Ca^{2+} is placed in a solution of 1 wt % Alg. At t = 0, a potential of 10 V is applied. (a) Photos showing the growth of an Alg gel layer around the disc for a Ca^{2+} concentration of 0.01 wt %. (b) Thickness of the Alg gel layer as a function of time for three Ca^{2+} concentrations. (c) Photo of the Alg gel around the disc for a Ca^{2+} of 1 wt % after 5 min of applying the potential. The scale bars in all images are 2 mm.

agarose) shrinks during the electrogelation process. For example, the disc-shaped agarose in Figure 3a can be seen to shrink in diameter from 5.5 to 3 mm over 5 min of applying the electric field. Such shrinkage is not seen at 1 wt % Ca^{2+}, as can be noted from Figure 3c. We hypothesize that the shrinking is due to the stresses exerted by the charged Alg gel on the agarose. That is, Alg chains in the low-Ca^{2+} gel layer would still retain sufficient anionic character and therefore attempt to move electrophoretically toward the anode, and this would exert compressive stress on the central agarose. At higher Ca^{2+}, the Alg chains in the gel layer would have negligible residual charge and would also be constrained by the additional cross-links. Thus, the Alg would not have the same electrophoretic tendency. Interestingly, we have found that the shrinkage of the core is reduced at higher agarose concentrations. Figure S1 in the Supporting Information (SI) section compares the shrinkage of cores made with 1, 2.5, and 5 wt % agarose. The core shrinkage is significant at 2.5 wt % and even more pronounced at 1 wt % agarose, but it is negligible at 5 wt % agarose. The likely explanation is that a stiffer agarose core will be able to resist the compressive stresses exerted by the Alg chains. Thus, if it is critical to preserve the mold dimensions, a 5 wt % agarose mold would be the better choice.

Effects of Variables on Gel Properties. We proceeded to vary the Ca^{2+} concentration in the agarose mold to see if it would affect the properties of the Alg gel (specifically, the rheological properties). In all cases, Alg gels were allowed to form for 5 min at 10 V around a cylindrical agarose mold, which was placed in a 1% Alg solution. Thereafter, the mold was removed, the Alg gels were cut into disks of diameter 20 mm, and then tested on a rheometer. Figure 4 shows a plot of

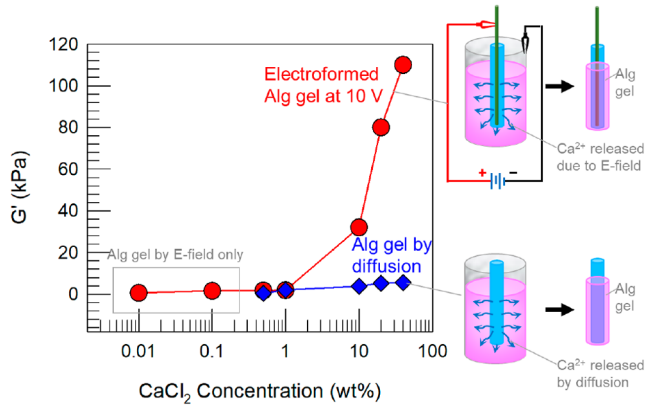


Figure 4. Comparing the rheology of Alg gels formed in the presence and absence of an electric field. The elastic modulus G' of the gels is plotted as a function of Ca^{2+} in the agarose mold. During electroformation, the gel arises due to electrically induced migration of Ca^{2+}, as shown by the top schematic. When the field is switched off and the Ca^{2+} is high, a gel can still form by simple diffusion of Ca^{2+} out of the mold, as shown by the bottom schematic. When the Ca^{2+} is low (0.01 or 0.1 wt %), the Alg gel forms only when the field is switched on.

the elastic modulus G' of the Alg gels against the Ca^{2+} concentration. In the absence of an electrical signal, there are no data points below 0.5 wt % Ca^{2+} because no gel is formed under these conditions. When the 10 V potential is applied, G' is 0.6 kPa for 0.01 wt % Ca^{2+} and 1.6 kPa for 0.1 wt % Ca^{2+}. G' then increases sharply as the Ca^{2+} is further increased. No significant change in G' is observed for the gels formed by simple diffusion (without an electric field), regardless of the

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Ca^{2+} in the mold. This means that the electric field enhances the gel properties. To show this further, frequency sweeps are plotted in Figure 5 for Alg gels formed at 10 wt % Ca^{2+} by

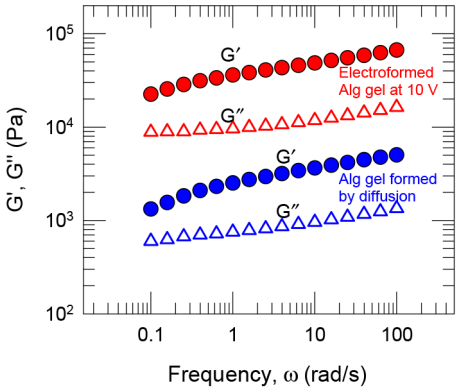

Figure 5. Frequency spectra of Alg gels made by electroformation or simple diffusion. The elastic modulus G' and the viscous modulus G'' are plotted against frequency for Alg gels created using molds with 10 wt % Ca^{2+}. Note the much higher moduli for the electroformed gel.

electrical signals and by simple diffusion. Both gels show elastic rheology ($G' > G''$, moduli nearly independent of frequency),[35,36] but the magnitude of G', which reflects the gel stiffness,[35] is about 10-fold higher for the electroformed gel. The higher G' of the electroformed gel indicates that it has a higher density of Ca^{2+} cross-links compared to the gel formed by diffusion.

Next, we varied the applied potential to see its effect on Alg gel formation. These tests were performed with agarose molds having 0.1 wt % Ca^{2+} and the solution containing 1% Alg. All other parameters were kept constant. Figure 6 shows the

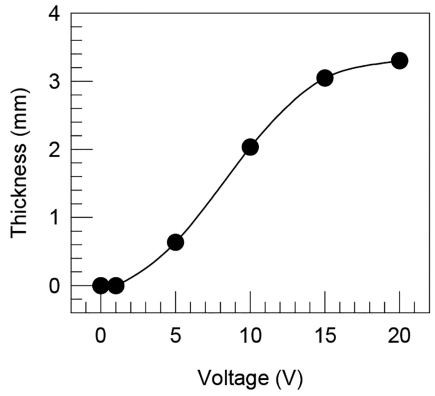

Figure 6. Thickness of Alg gels as a function of the applied potential. The gels were formed around agarose molds that contained 0.1 wt % Ca^{2+}.

thickness of the Alg gel after 5 min at specific voltages. We note that the thickness is zero at 1 V, then increases with increasing voltage, and finally saturates around 20 V. The increase in gel thickness with voltage is expected because a higher voltage increases the electrophoretic velocity of Ca^{2+} ions, allowing the ions to migrate farther from the mold and hence gel a larger volume of Alg chains.

Gels with Specific Morphologies, Payloads, and Patterns. Our inside-out technique, i.e., gelation starting from the core and extending outward, can be used to grow sequential Alg gels. This can be used to form concentric multilayer structures, as illustrated in Figure 7. We have incorporated fluorescent (Fl) nanoparticles (NPs) in different layers to distinguish them. First, a cylindrical agarose mold with 0.1 wt % Ca^{2+} is created. This is placed in an Alg solution (1 wt %) that contains dispersed green-fluorescent NPs (0.05 wt %). A potential of 10 V is applied for 30 s to form the first Alg gel layer, which will have the NPs immobilized in it (Figure 7a). This procedure is then repeated with a second Alg solution with no NPs (Figure 7b) and finally with a third Alg solution containing 0.05 wt % of red-fluorescent NPs (Figure 7c). The fluorescence micrograph of the final cross-section shows the green-colorless-red sequence of Alg layers (from the center proceeding outward), as expected. Note that this process can be extended even further. As long as there are Ca^{2+} ions left in the agarose core, we can grow a fresh Alg gel layer on the periphery. Also, the technique is versatile and rapid. Each layer is formed in just 30 s, and thus it is possible to rapidly build multiple, distinct layers.

Our electrogelation process can be done under mild and biologically benign conditions. It can be implemented with the agarose mold in a solution of Alg made using a physiological buffer, and the temperature can be maintained at 25 or 37 °C. This means that biomolecular or biological payloads in the Alg solution, such as enzymes or cells, can be incorporated into the corresponding Alg gel. To illustrate this point, we demonstrate the encapsulation of bacteria in Alg gel layers. Two strains of genetically engineered *Escherichia coli* bacteria were used: one that expressed a red-fluorescent protein (RFP) and the other that expressed green-fluorescent protein (GFP). Pellets of these bacteria were combined with the Alg in phosphate-buffered saline (PBS). An agarose mold in a disc shape (1 cm diameter, 5 mm height) bearing 0.1 wt % Ca^{2+} was used as the core. The procedure was similar to that in Figure 7, and a total of three layers of Alg gel were formed successively around the agarose core. The disc was then removed and placed in growth media for the bacteria to proliferate.

The schematic of the final disc and its photo are shown in Figure 8. Prior to capturing the photo, it was found that the core could be easily pushed out of the disc center by mechanical action, and thus the photo shows a disc with an empty core. The fluorescence micrograph of the disc cross-section correlates with the schematic and shows three concentric layers around the core. Layer 1 (innermost layer) has a bright-red fluorescence as it contains *E. coli* that express RFP. Layer 2 is a blank Alg gel and therefore shows no fluorescence. Finally, layer 3 (outermost layer) has a bright-green fluorescence due to the GFP-expressing *E. coli* in that Alg gel. The strong fluorescence signals confirm that the bacteria are able to proliferate in the Alg gel layers. On the whole, the results in Figure 8 show that our technique is biocompatible and can be used for cell encapsulation. The ability to place different types of cells in adjacent hydrogel layers could be important in studying the communication between different cell types[37] or in building faithful models of various tissues.[38]

In the experiments thus far, the Alg gel was electroformed everywhere around the original agarose mold. We now show that, by using a hydrophobic coating on the mold, we can dictate the gel growth to occur only in specific regions. For this proof of concept, we have used a commercially available hydrophobic coating ("Rust-Oleum NeverWet spray"). In the first case (Figure 9A), we start with a flat sheet of agarose containing 0.1 wt % Ca^{2+} with dimensions of 6 cm × 1 cm and

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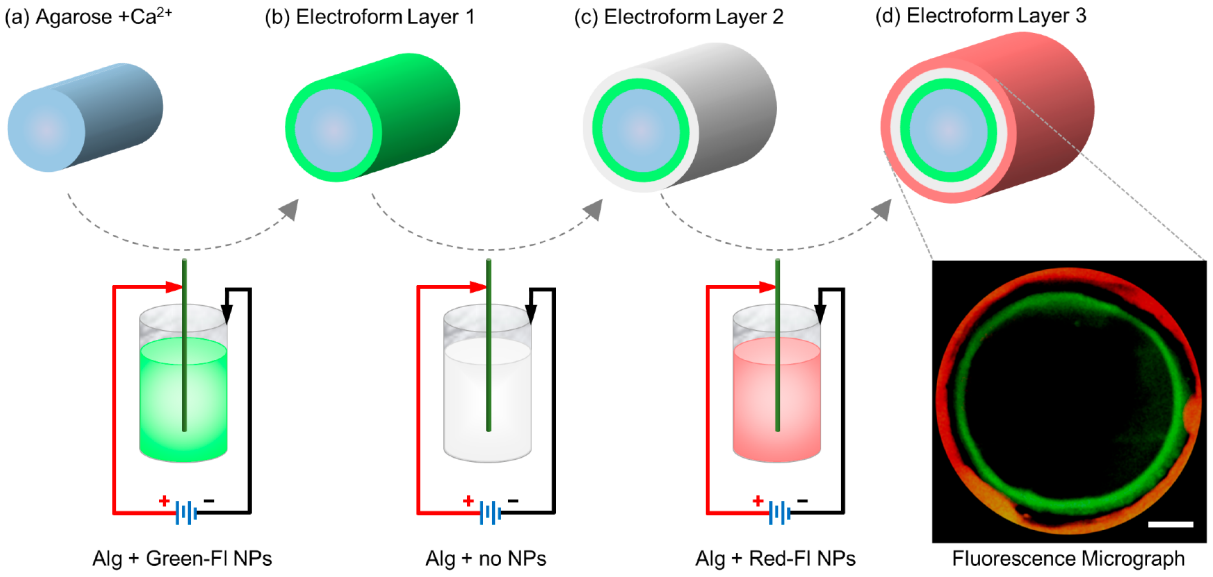

Figure 7. Electroformation of multiple, distinct Alg gel layers concentrically around an agarose core. The Ca^{2+}-containing cylindrical agarose mold (a) is coated with successive layers of Alg gels containing (b) green-fluorescent nanoparticles (green-Fl NPs); (c) no NPs; and (d) red-fluorescent NPs. A fluorescence micrograph of the tube cross-section shows the multiple layers of the final structure. Scale bar: 1 mm.

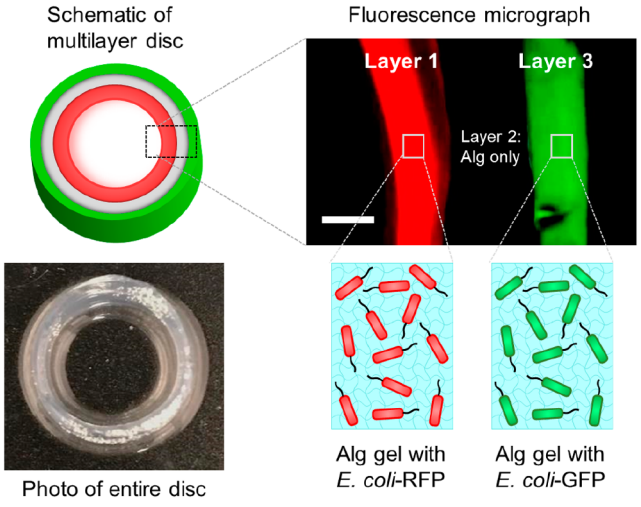

Figure 8. Electroformed Alg gel layers encapsulating two distinct strains of bacteria. Using a scheme similar to Figure 7, successive layers of Alg gel are grown concentrically around an agarose disc, which is then removed after synthesis. Layer 1 has a strain of *E. coli* that express a red-fluorescent protein (RFP). Layer 2 is a bare Alg gel. Layer 3 has another strain of *E. coli* that express green-fluorescent protein (GFP). The fluorescence micrograph is a zoomed-in view of the disc cross-section showing the distinct layers. Scale bar: 1 mm.

a thickness of 8 mm. The hydrophobic coating is applied selectively over a central portion of the sheet (1.2 cm wide, extending across the width), as shown in Panel 1. When the electric field is turned on, the Alg gel (pink color due to acid red 52 dye) grows vertically over the regions not covered by the hydrophobic coating. This is shown schematically in Panel 2 and by the photo in Panel 3. Over the rest of the mold, the coating prevents the Ca^{2+} ions and the Alg chains from contacting each other to form a gel.

Next, we demonstrate the patterned growth of Alg gels using the same hydrophobic coating (Figure 9B). We cut aluminum foil in the shape of the letter "M" and place this on the agarose mold. The hydrophobic coating is then sprayed onto the mold. When the foil is removed, the mold is covered with the coating everywhere except for the "M" region (Panel 1). The presence of the coating could be verified from the fact that water does not wet the coated region, i.e., the contact angle of water drops placed on this region exceeds 90°. Next, the electric field is applied for 5−7 min, and we see that the Alg gel selectively grows only on the uncoated region, i.e., in the pattern of the "M" (Panels 2 and 3). The feature sizes in the pattern here are in the millimeter scale, but finer feature sizes can be obtained using lithographic masks and/or a more sophisticated coating.

Comparison to Other Techniques. The electroformation technique introduced in this paper is easy to implement in any lab. It requires only a few components, such as a power source and electrodes, which are all quite inexpensive. In contrast, 3-D printing requires investment in expensive equipment, as well as associated software. Many manufacturers of 3-D printers also require or emphasize the use of proprietary "bio-inks" with these printers. Our method, on the other hand, simply utilizes alginates of known composition to make our gels. Thus, our method has some advantages, and as discussed above, it is particularly suited to making certain structures such as long, hollow tubes. Other 3-D structures are also possible, and some examples are shown in Figure S2 in the SI section. Figure S2a shows a 20 cm-long alginate tube, which is much longer than the one in Figure 2. This long tube can be made in less than 30 min, which is the same amount of time as needed for a short tube. In 3-D printing, making a long tubular structure is often problematic (since tall, hollow structures will tend to sag) and time-consuming. Also, our electroformed alginate gels are at least as robust (if not more so) than comparable gels made by 3-D printing. With regard to the resolution of our technique, we also note that finer resolution is possible by varying the size of the agarose template and the Ca^{2+} loading. As an example, Figure S3 (SI) shows the growth of a micron-scale alginate layer around a 2 mm diameter agarose template. Thus, it is possible to achieve resolution on the order of tens of microns with our technique.

DOI: 10.1021/acsami.9b12575
ACS Appl. Mater. Interfaces 2019, 11, 37103−37111

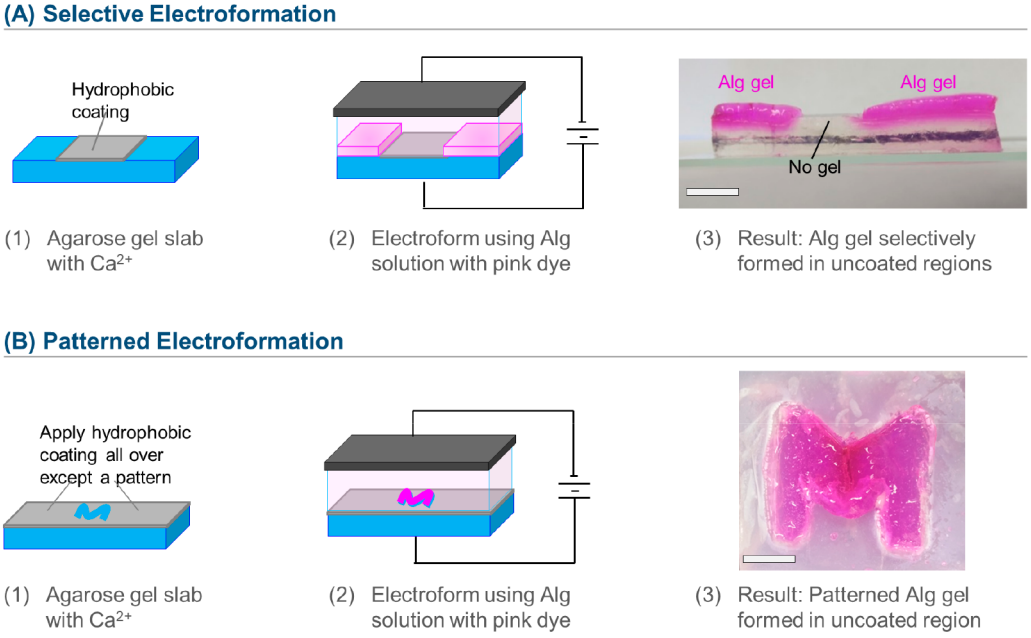

Figure 9. Electroformation of Alg gels over selected areas or in a pattern. (A) Selective electroformation is achieved using a commercial hydrophobic coating. (1) Coating is applied onto a central region of the agarose mold. (2) During electroformation, the Alg gel (dyed pink) grows only on the uncoated side regions. (3) Photo (side view) shows the selective growth of the Alg gel. (B) Variation of the above allows the gel to be formed in a pattern. (1) Coating is applied everywhere on the agarose mold except on the region corresponding to the letter "M". (2) Upon electroformation, the Alg gel (dyed pink) grows only over the uncoated "M" region. (3) Photo shows the Alg gel in the desired pattern. Scale bars: 1 cm.

CONCLUSIONS

We have described a new technique whereby an Alg gel forms around an agarose mold only when an electric field is switched on. This is accomplished by loading the mold with Ca^{2+} and placing it in an Alg solution, followed by application of a DC field (~10 V). The Ca^{2+} cations and the anionic Alg chains migrate via electrophoresis and meet at the mold surface. Cross-linking of the Alg by Ca^{2+} leads to a transparent and robust gel. The gel grows rapidly and steadily (at about 0.8 mm/min) in an outward direction from the mold surface for low Ca^{2+} concentrations. The utility of this technique is demonstrated by our ability to create Alg gel structures that are not readily accessible through traditional methods. For example, we have electroformed multilayer disks and tubes, which have several concentric layers, each with a unique payload. We have shown that biological cells can be encapsulated in individual Alg layers. Finally, we have illustrated the ability to electroform Alg gels in specific patterns by using a hydrophobic coating to confine gel growth to specific areas. On the whole, electroformation could offer a viable alternative to 3-D printing for custom-manufacturing of hydrogels, especially for biomedical applications. Unlike 3-D printers, which are cumbersome and expensive, the setup for electroformation requires only a few components that should be readily available to any lab. Thus, any lab can be enabled to do 3-D manufacturing of gels without the need for an actual 3-D printer.

EXPERIMENTAL SECTION

Materials and Chemicals. Alginate (medium molecular weight), calcium chloride dihydrate, and agarose (type 1-A, low electro-endosmosis, melting temperature of ~88 °C) were obtained from Sigma-Aldrich. Graphite pencil lead (Pentel Super Hi-Polymer, 0.9 mm) for use as the electrode was purchased from Staples, and Rust-Oleum "NeverWet" spray was purchased from The Home Depot. Methylene blue dye was purchased from Sigma-Aldrich, and acid red 52 dye was obtained from TCI America. Red (diameter of ~500 nm) and green (diameter of ~100 nm) fluorescent latex nanoparticles (NPs) were purchased from Polysciences Inc. All chemicals were used as received.

Agarose Gel Preparation. Agarose gels were prepared by first dissolving weighed amounts of $CaCl_2$ into DI water and heating the solution to above 80 °C. Subsequently, 2.5 wt % of agarose was added to the solution, and the mixture was heated until the agarose completely dissolved. The hot solution was then poured into test tubes (1.2 cm diameter, 7.5 cm height) with the graphite electrode embedded and allowed to cool down to room temperature.

Experimental Setup. For the setup shown in Figure 1, a DC power source (Agilent E3612A) and a beaker (diameter 5 cm) wrapped on its inside with Al foil were used. The cylindrical agarose gel containing the graphite electrode was placed in the center of the beaker and filled with 90 mL of 1 wt % Alg. The positive terminal of the power source (anode) was connected to the graphite electrode and the negative terminal (cathode) to the Al foil.

Setup for Kinetic Study. For the kinetic study shown in Figure 3, an agarose gel disc (5.5 mm diameter, 4 mm height) with graphite electrode at its center was placed in a Petri dish (diameter 50 mm) filled with 10 mL of 1 wt % Alg and wrapped on its inside with Al foil. The setup was monitored using a high-resolution camera. A bias of 10 V was applied, and bright-field images were taken at regular intervals. The images were analyzed using ImageJ software.

Rheological Measurements. Alg gels were grown around cylindrical agarose cores, and pieces of these gels were cut into disks of 20 mm diameter. Rheological studies were performed on these disks using an AR2000 stress controlled rheometer (TA Instruments) using a 20 mm parallel plate geometry at 25 °C. Dynamic frequency sweeps were performed in the linear viscoelastic region of each sample as determined by prior dynamic stress sweeps.

Multilayer Cylinder (with NPs). Three Alg solutions were prepared (all with 1% Alg by weight). Into solution-1, green fluorescent NPs (0.05 wt %) were added, and into solution-3, red fluorescent NPs (0.05 wt %) were added. Solution-2 had just the Alg

DOI: 10.1021/acsami.9b12575
ACS Appl. Mater. Interfaces 2019, 11, 37103−37111

without any NPs. An agarose cylinder (6 mm diameter, 6 mm long) loaded with 0.1 wt % $CaCl_2$ was first placed in solution-1, then in solution-2, and finally in solution-3 (see Figure 7). Each electrogelation step was carried out for 30 s at 10 V. The cylinder was washed with DI water in between each step. Images of the final cylinder were taken under a fluorescence microscope (Zeiss Axiovert 135 TV). To image green fluorescence, a band-pass excitation filter at 450−490 nm and a band-pass emission filter at 515−565 nm were used. To image red fluorescence, a band-pass excitation filter at 530−585 nm and a long-pass emission filter at 615 nm were used. The images were overlaid using ImageJ software to visualize both colors simultaneously.

Multilayer Disc (with Bacteria). Two strains of *E. coli* were used in the experiments: W3110+dsRed and W3110+GFP. The former was engineered to overexpress a red fluorescent protein (RFP) called dsRed, while the latter overexpressed a green fluorescent protein (GFP). Both strains were grown in Luria broth (LB) medium at 37 °C and on a shaker at 250 rpm. Cells were reinoculated at a 1:100 dilution from overnight cultures and induced to grow for 2 h to the mid-logarithmic level (optical density 600 of 0.6). Cells were then collected by centrifugation at 7000 rpm for 10 min, and pellets were resuspended in 1 mL of 1× phosphate-buffered saline (PBS). Resuspended cells were mixed with 5 mL of a 1.5 wt % Alg solution. Three Alg solutions were prepared. Solution-1 contained the +RFP cells, solution-3 contained the +GFP cells, and solution-2 contained no cells. An agarose disc (1 cm diameter, 5 mm height) loaded with 0.1 wt % $CaCl_2$ was first placed in solution-1, then in solution-2, and finally in solution-3 (see Figure 8). Each step was carried out for 1 min at 10 V, and the gel was rinsed three times with 1× PBS between each step. The resulting disc with cells was incubated in LB medium at 37 °C for 4 h to induce cell growth. Upon removal from the medium, the core was pushed cleanly out of the disc center, thereby yielding a hollow disc. Images of the disc were taken under a fluorescence microscope as above.

Patterned Gel Growth on a Surface. A slab of agarose containing 0.1 wt % Ca^{2+} with dimensions of 6 cm × 1 cm × 8 mm was used in the experiments. The slab contained an embedded graphite plate (dimensions: 6 cm × 1 cm × 8 mm), which served as the anode. The hydrophobic coating (Rust-Oleum "NeverWet") was sprayed over a central portion of the sheet (1.2 cm wide), as shown in Figure 9. A 1% Alg solution with 0.1 wt % of acid red 52 dye was then prepared. The agarose slab was placed in 10 mL of the above solution in a beaker (diameter 10 cm) coated on its inside with Al foil, which served as the cathode. A height of 2 cm was maintained between the agarose and the bottom of the beaker. Gel growth occurred vertically downward from the surface of the agarose when the electric field was turned on.

■ ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/acsami.9b12575.

Effect of agarose concentration on the core-shrinking phenomenon observed during electroformation (Figure S1); electroformed alginate (Alg) gels in various shapes and geometries (Figure S2); electroformed alginate (Alg) gel around an agarose core containing 0.01 wt % Ca^{2+} as monitored by optical microscopy (Figure S3) (PDF)

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Notes

The authors declare no competing financial interest.

■ ACKNOWLEDGMENTS

This work was partially supported by grants from NIST (70NANB12H238) and NSF (DMREF 1435957). The authors acknowledge the assistance of Narendranath Bhoksham from the Bentley lab for some of the experiments. The authors also acknowledge helpful discussions with Prof. Greg Payne.

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DOI: 10.1021/acsami.9b12575
ACS Appl. Mater. Interfaces 2019, 11, 37103−37111

Supporting Information for:

Rapid Electroformation of Biopolymer Gels in Prescribed Shapes and Patterns: A Simpler Alternative to 3-D Printing

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Figure S1. Effect of agarose concentration on the core-shrinking phenomeon observed during electroformation. The agarose core was loaded with 0.01 wt% Ca^{2+} in each case and placed in a solution of 1 wt% Alg. Electroformation was done at a voltage of 10 V for 5 min, much like in Figure 3. Images are shown at the initial stage (left) and after electrodeposition (right). Note that the clear layer around the core on the right is the Alg gel. The core shrinks in the case of agarose concentrations of (a) 1 wt% and (b) 2.5 wt%, but not in the case of (c) 5 wt%.

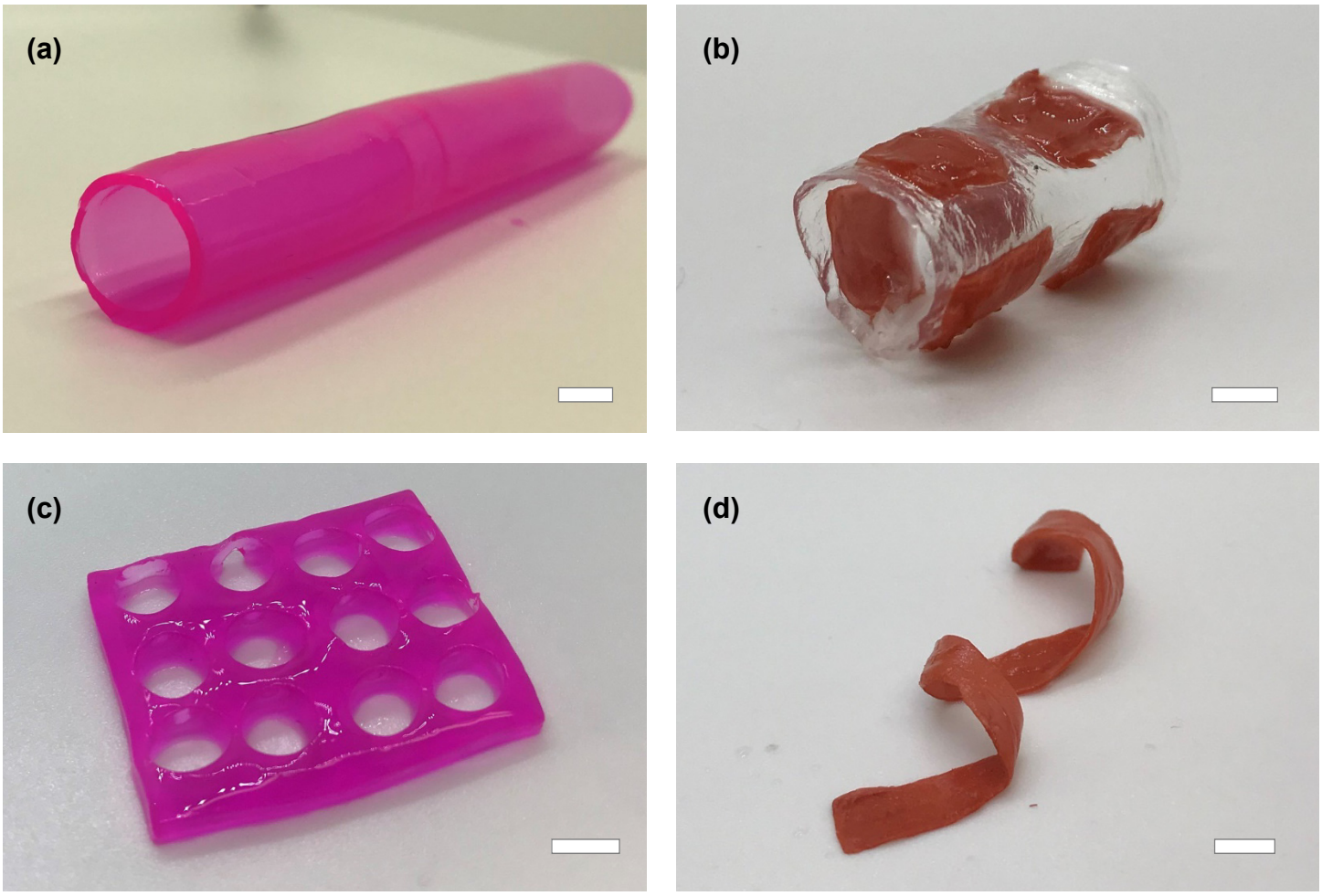

Figure S2. Electroformed alginate (Alg) gels in various shapes and geometries. Experimental details on the synthesis of these gels are given below on page S-5. (a) A long Alg tube with an inner diameter of 12 mm and a length of ~ 20 cm. (b). Alg tube (12 mm diameter, ~ 25 mm length) with a second Alg layer deposited as square patches (5 × 5 mm). (c) Cuboidal Alg gel (length and width ~ 3 cm and thickness 7 mm) with round holes (5 mm diameter). (d) Helical strip of Alg gel with a thickness of 500 μm, width of 8 mm, and length ~ 3 cm. The helix diameter is ~ 12 mm and its pitch is ~ 15 mm. Note that the pink color of the gels in (a) and (c) is due to acid-red 52 dye while the brown color in (b) and (d) is due to iron-oxide pigment particles. Scale bars are 5 mm in (a), (b) and (c), and 10 mm in (d).

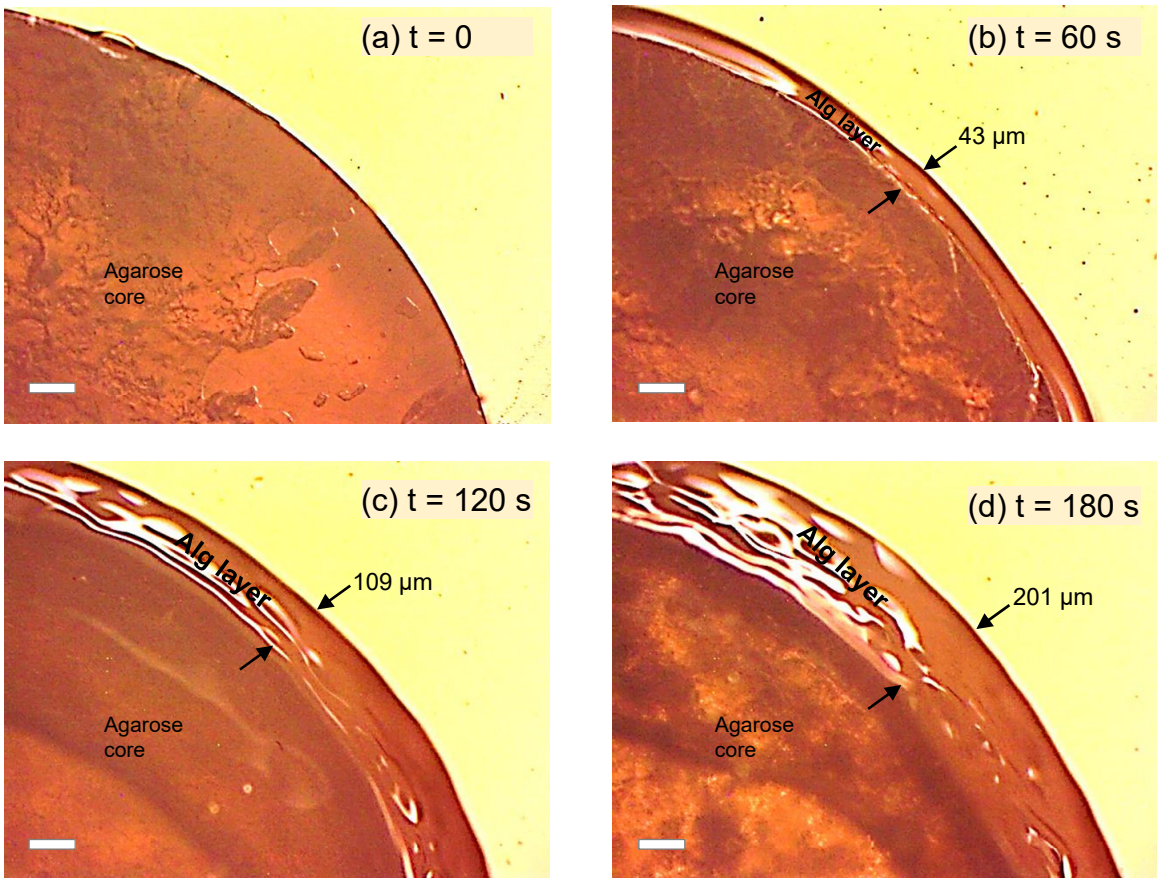

Figure S3. Electroformed alginate (Alg) gel around an agarose core containing 0.01 wt% Ca^{2+} as monitored by optical microscopy. Experimental details on this procedure are given below on page S-5. The images in (a) to (d) show the growth of the alginate gel layer with time, and the thickness of this gel layer can be tuned on the order of microns. Scale bars are 100 μm in all cases.

Experimental Details for Figures S2 and S3

Preparation of long alginate tube (Figure S2a):

The Alg tube was prepared using the same general method as described in the main paper. For the specific tube shown here, the agarose core was loaded with 5 wt% $CaCl_2$. This core was immersed in a 2 wt% Alg solution containing 100 mM of acid-red 52 dye, and electroformation was done for 10 min. The structure was then removed from the Alg solution and placed in 0.5 M $CaCl_2$ for 15 min to ensure full crosslinking of the Alg. The cylinder ends were then cut and the agarose core could be removed by gently pushing from one end.

Preparation of alginate tube with square patches (Figure S2b):

First, an Alg tube was made using a cylindrical agarose core loaded with 0.5 wt% $CaCl_2$ using the procedure laid out in Figure 2 of the main paper. This tube was washed with DI water and wrapped with a piece of plastic wrap with square cutouts that served as a template. The wrapped tube was then submerged in a 1 wt% Alg solution that contained 1 wt% of iron-oxide powder (added for visualization). After electroformation of the second layer for 1 min, the plastic wrap was removed. Both ends of the cylinder were cut with a razor blade and the agarose core was removed by gently pushing from one end.

Preparation of cuboidal alginate gel with round holes (Figure S2c):

A piece of agarose gel (25 x 25 x 10 mm) was cut from a flat sheet. The gel was loaded with 0.1 wt% $CaCl_2$. Plastic straws with 5 mm diameter and 2 cm height were inserted into this gel in the form of a well-spaced array. This gel with the straws was then submerged partially (up to about 5 mm in the thickness-dimension) in a 1 wt% Alg solution containing 100 mM of acid-red 52 dye. Electroformation was then done for 3 min. The resulting structure was removed and washed with DI water. The agarose mold was then removed by cutting the edges of the resulting gel with a razor blade, leaving the cuboidal alginate gel with round holes.

Preparation of helical alginate-gel strip (Figure S2d):

An agarose gel cylinder containing no $CaCl_2$ was coated with the Rust-Oleum Never-Wet spray used in Figure 9. A helical strip of desired dimensions was then 'painted' on this cylinder using a 5 wt% agarose paste containing 5 wt% $CaCl_2$ (the paste was at a point right before the agarose gelled). After the helical strip set into a gel, the structure was submerged in a 1 wt% Alg solution that contained 1 wt% of iron-oxide powder (added for visualization). Upon electroformation for 1 min, the Alg gel formed only over the pattern drawn with agarose paste, resulting in a helical strip of Alg gel. The strip could be detached easily from the coated core, and thereafter, the agarose gel used for the helical pattern was removed from the Alg gel strip using a tweezer.

Setup for kinetics study (Figure S3):

An agarose gel disc (2 mm diameter, 10 mm height) loaded with 0.01 wt% $CaCl_2$ was first prepared, and a graphite electrode was inserted into its center. This gel was placed in a Petri dish filled with a 1 wt% Alg solution and wrapped on its inside with Al foil. The setup was monitored using an inverted optical microscope (Zeiss Axiovert). A bias of 10 V was applied and bright field images were taken at regular intervals. The images were analysed using the ImageJ software.