Heterogeneous Films of Ionotropic Hydrogels Fabricated from Delivery Templates of Patterned Paper

Paul J. Bracher, Malancha Gupta, Eric T. Mack, and George M. Whitesides*

Department of Chemistry and Chemical Biology, Harvard University, 12 Oxford Street, Cambridge, Massachusetts 02138

ABSTRACT The use of delivery templates makes it possible to fabricate shaped, millimeter-thick heterogeneously patterned films of ionotropic hydrogels. These structures include two-dimensional (2-D) patterns of a polymer cross-linked by different ions (e.g., alginic acid cross-linked with Ca^{2+} and Fe^{3+}) and patterns of step gradients in the concentration of a single cross-linking ion. The delivery templates consist of stacked sheets of chromatography paper patterned with hydrophobic barriers (waterproof tape, transparency film, or toner deposited by a color laser printer). Each layer of paper serves as a reservoir for a different solution of cross-linking ions, while the hydrophobic barriers prevent solutions on adjacent sheets from mixing. Holes cut through the sheets expose different solutions of cross-linking ions to the surface of the templates. Films with shaped regions of hydrogels cross-linked by paramagnetic ions can be oriented with a bar magnet. Variations in the concentrations of cations used to cross-link the gel can control the mechanical properties of the film: for single alginate films composed of areas cross-linked with different concentrations of Fe^{3+}, the regions cross-linked with high concentrations of Fe^{3+} are more rigid than regions cross-linked with low concentrations of Fe^{3+}. The heterogeneous hydrogel films can be used to culture bacteria in various 2-D designs. The pattern of toxic and nontoxic ions used to cross-link the polymer determines the pattern of viable colonies of *Escherichia coli* within the film.

KEYWORDS: hydrogels • biofilms • alginate • carrageenan • patterning bacteria

INTRODUCTION

This article describes the design of delivery templates useful in the fabrication of millimeter-thick heterogeneous films of ionotropic hydrogels. These structures include films where a pattern of one hydrogel is surrounded by another hydrogel ("gel-in-gel" structures) and films that contain a step gradient in the concentration of the cross-linking agent. The procedure described here provides a simple method to produce mixed films of ionotropic hydrogels for use in biological studies. We demonstrate that the pattern of the components of the hydrogel can control the growth of *Escherichia coli* within the films.

Ionotropic hydrogels form when ions of one charge cross-link polymers of the opposite charge. The most common examples of ionotropic hydrogels are the gels formed when multivalent cations (e.g., Ca^{2+} or Fe^{3+}) cross-link anionic polysaccharides such as alginic acid (AA) and carrageenan (CG) (1−3). Alginate cross-linked with Ca^{2+} (Ca^{2+}-AA) is nontoxic to many mammalian cells and can be used for tissue engineering, cell encapsulation, controlled release of drugs, and wound dressings (4−8).

The ability to design and fabricate heterogeneous materials such as films and functionalized surfaces is an important tool for research in cell biology and materials chemistry (9). For example, the migration of bacteria and other cells can be controlled by gradients in the concentration of molecules (chemotaxis) (10, 11) or gradients in the mechanical rigidity of the substrate (durotaxis) (12, 13). Cellular interactions within hydrogels can be studied using heterogeneous films that comprise patterned regions of distinct cell types (14, 15).

Several procedures can generate hydrogels that are heterogeneous on the scale of micrometers using microfluidics and microcontact printing. For example, microfluidic devices that generate gradients have been used to produce gels of poly(ethylene glycol) (16) and poly(acrylamide) (17) with a gradient of cross-linking densities. Liu and Bhatia and Zguris et al. combined multistep lithography and photopolymerization to produce heterogeneous structures of poly(ethylene glycol) within microchannels (14, 15). The limitations of the technique are that it requires multiple steps of lithography, the photomasks must be aligned for each exposure, and UV light can harm cells.

Grzybowski and co-workers fabricated films of gelatin with multiple-colored precipitates by soaking an agarose stamp in a mixture of inorganic salts ($FeCl_3$, $CuCl_2$, and $CoCl_2$) and bringing the stamp in contact with a dry film of gelatin doped with potassium hexacyanoferrate (18). The cations from the stamp diffused into the gels at different rates and reacted with the hexacyanoferrate anions in the gel to form colored precipitates. Cohen and co-workers used a robotic printing system to fabricate heterogeneous structures of AA (19). They printed solutions of alginate for which cross-linking with $CaSO_4$ had been initiated prior to deposition.

Heterogeneous surfaces can be used to study biofilms. Biofilms are structurally complex communities of bacterial cells surrounded by hydrated extracellular polymeric matri-

* Corresponding author. E-mail: gwhitesides@gmwgroup.harvard.edu.
Received for review May 18, 2009 and accepted July 17, 2009

DOI: 10.1021/am900340m

ces (20). The polymeric matrices excreted by the cells typically comprise polysaccharides, proteins, and nucleic acids (21). The formation of biofilms can occur on (and pose problems to) a variety of surfaces, such as glass (22), metals (23, 24), and plastics (25). These include implanted medical devices such as venous catheters, urinary catheters, contact lenses, prosthetic joints, prosthetic heart valves, and pacemakers (26–28). Bacterial adhesion is the first stage in the formation of biofilms (21). Inhibition of bacterial adhesion on hydrogels is important for the development of hydrogel coatings for medical implants (29) and contact lenses (30, 31).

We define a *delivery template* as a patterned material that both stores a reagent or substance and delivers it, in a designated pattern, to a second medium. In previous work, we patterned a water-impermeable barrier (e.g., tape or printed toner) onto single sheets of paper—either photolithographically, by hand, or with a color laser printer—to produce templates for the fabrication of shaped films composed of a single type of ionotropic hydrogel (32–34). The paper transferred solutions of multivalent ions, in a pattern, to solutions of anionic polymers applied to the surface of the template. In this paper, we report a method for the fabrication of heterogeneous films of ionotropic hydrogels and demonstrate that the pattern of metal ions used to cross-link the gels can control the growth of bacteria within the films.

EXPERIMENTAL DESIGN

Our strategy to generate heterogeneous films composed of distinct regions of ionotropic hydrogels was to create templates for the delivery of cations by stacking patterned layers of paper with interstitial waterproof barriers (Figure 1). The small thicknesses of the paper, tape, and printed layers of toner allowed us to cut and stack these materials to form templates with complex patterns in the *xy* plane and only slight (≤0.2 mm) unevenness in the *z* direction. Each layer of paper stored a different type of cross-linking cation, while the water-impermeable barriers prevented solutions on adjacent layers of the paper from mixing.

The method consists of three general steps: (i) constructing the delivery template, (ii) pouring a solution of un-cross-linked polymer onto the template to form the patterned hydrogel film, and (iii) isolating the film from the template. The delivery templates are layered structures of patterned paper and hydrophobic barriers used to control the delivery of multivalent cations to a solution of un-cross-linked polymer. A typical delivery template consists of five layers (Figure 1e, oblique view; Figure S1 in the Supporting Information, cross-sectional view). Layer 1, the bottom layer, is a flat, sturdy waterproof surface, such as a plastic Petri dish. Layer 2 is an unpatterned sheet of chromatography paper that serves as a reservoir for one solution of cross-linking ions. Layer 3 is a waterproof layer of toner patterned on the underside of another sheet of paper, layer 4. The toner serves as a waterproof barrier that prevents ions on the two sheets of paper from mixing. Layer 4 serves as a reservoir for a second solution of cross-linking ions and has shaped holes that expose regions of layer 2 to the surface of the template. Layer 5, the top layer of the template, is a sheet of transparency film: a hydrophobic barrier that controls the shape of the perimeter of the hydrogel film.

We selected chromatography paper as the material to store and deliver the solutions of multivalent ions because it is absorbent, flat, thin, biocompatible, inexpensive, and com-

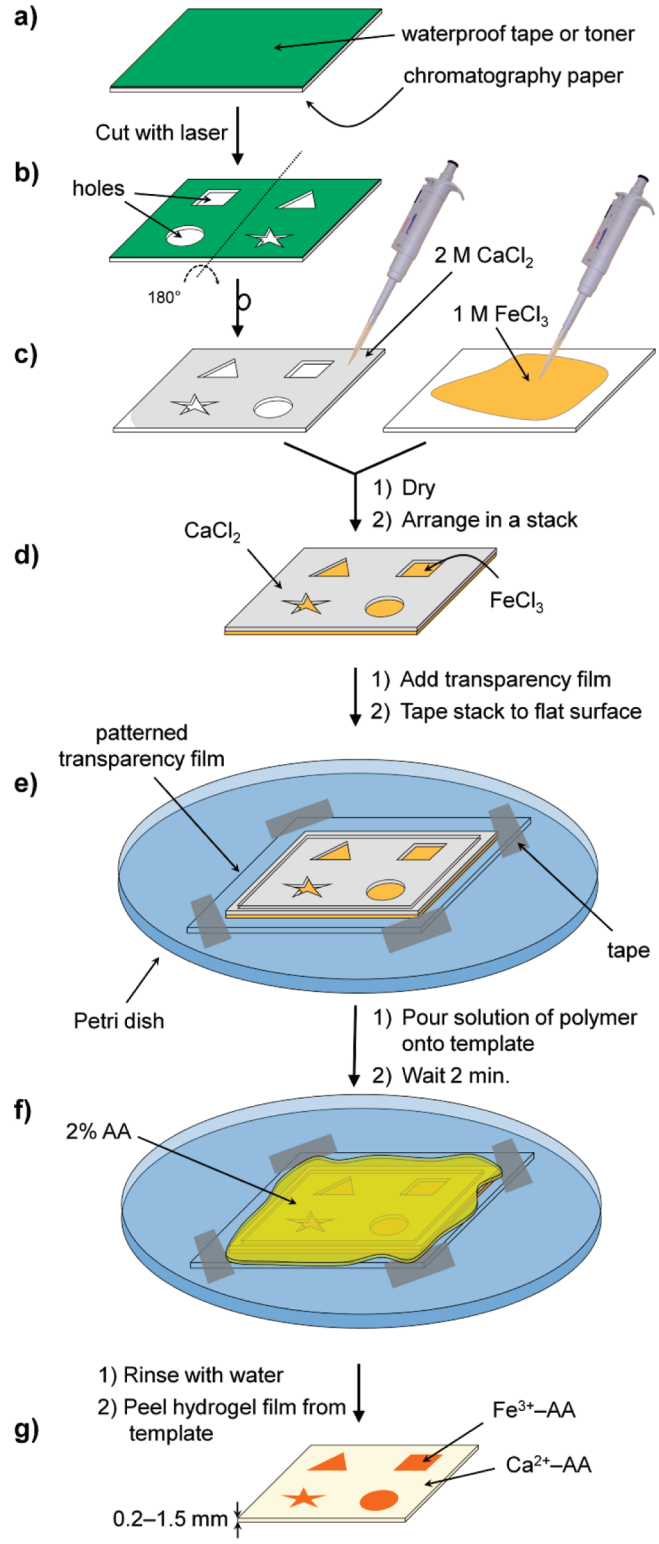

FIGURE 1. Steps for the fabrication of shaped, heterogeneous films of ionotropic gels made with different cross-linking ions. A sheet of Whatman No. 1 chromatography paper with a layer of color laser toner printed on one side (a) was patterned with a laser cutter (b). This patterned sheet and a new, unpatterned sheet were wetted with different solutions of cross-linking ions (c), dried, and stacked (d). A sheet of transparency film patterned with a laser cutter was placed on top of the paper to control the shape of the perimeter of the film, and the template was taped down to a Petri dish (e). A 2% solution of AA poured onto the template (f) gelled into a single heterogeneous film that was rinsed with water and peeled from the template with a metal spatula (g).

mercially available. In general, paper is a convenient material to use as a template because there are many common tools and products (e.g., printers, cutters, and adhesives) designed specifically for use with it. To control the delivery of solutions of cross-linking ions from the templates, we created two-dimensional (2-D) water-impermeable barriers by applying waterproof adhesive tape to the sheets of paper or by printing a continuous layer of hydrophobic toner onto the paper with a standard color laser printer. After it was heated to seal any cracks or holes, the toner (a mixture of polyester resin, pigments, wax, and amorphous silica) (35) blocked the passage of aqueous solutions between adjacent layers of paper. Toner was our preferred hydrophobic barrier because it formed a layer that was thinner than tape. Once a piece of paper was backed with a waterproof layer, we used an automated laser cutter to cut patterns through the sheet. These shaped holes exposed the top surface of a stack of papers to the underlying layer (Figure 1d). Waterproof tape or a sheet of transparency film with a hole cut in it controlled the shape of the perimeter of each film (Figure 1e).

RESULTS AND DISCUSSION

Production of Gel-in-Gel Structures Containing Different Cross-Linking Ions. Figure 1 summarizes the procedure used to make single films of a polymer cross-linked with different multivalent ions. The general procedure entailed printing of a continuous layer of toner onto one side of a sheet of paper and use of a laser cutter to cut shapes in it. This sheet was turned over, wet with a solution of cross-linking ions, dried, and pressed against a second (unpatterned) sheet of paper that contained a different cross-linking ion. In this configuration, once the aqueous solution of polymer came in contact with the template, the layer of toner sandwiched between the two sheets of paper prevented the salts from mixing. A solution of polymer (e.g., 2% AA) poured onto the surface of the paper formed films with a composition that corresponded to the pattern of the template. The top surface of the layered paper construct was effectively a new 2-D template with precisely shaped regions of two different cross-linking ions. A sheet of patterned transparency film placed on top of the stack of paper determined the shape of the perimeter of the film. This method could be extended to incorporate multiple regions cross-linked by different cations by cutting the bottom layer into several noncontiguous pieces and wetting each piece with a different solution of ions.

Figure 2a is an image of a film made by this method: a continuous film of AA with shapes of Fe^{3+}-AA inside a field of Ca^{2+}-AA. The top layer of the template used to make the film contained $CaCl_2$, and the bottom layer contained $FeCl_3$. The hydrogel film formed within 2 min when a 2% solution of sodium alginate in water came in contact with the two-layered paper template. A rinse with water removed excess (uncross-linked) alginate, and the gentle prying action of a metal spatula assisted in peeling of the film from the paper. The thickness of the Ca^{2+}-AA portion of the gel was 0.95 ± 0.19 mm, and the thickness of the Fe^{3+}-AA portion of the gel was 1.12 ± 0.08 mm. In an analogous experiment, we made the inverse pattern (shaped structures of Ca^{2+}-AA surrounded by Fe^{3+}-AA; Figure 2b) by using the same template and switching the solutions used to wet the two sheets of paper; the top layer contained $FeCl_3$, and the bottom layer contained $CaCl_2$. Heterogeneous films of ι-CG, another polymer commonly used to form ionotropic hydrogels, could be made by the same process (Figure 3).

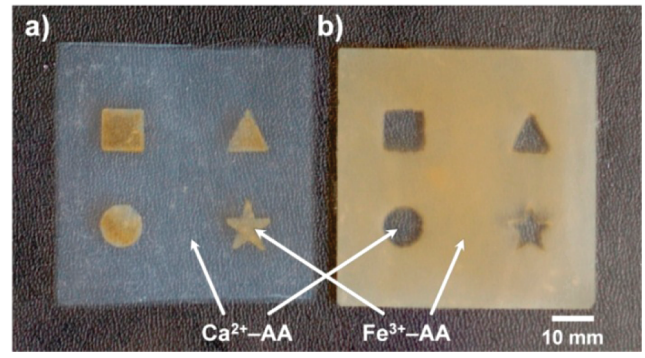

FIGURE 2. Continuous single films of AA cross-linked with different multivalent cations: (a) alginate film formed with shapes of Fe^{3+}-AA surrounded by Ca^{2+}-AA; (b) "inverse" film with shapes of Ca^{2+}-AA surrounded by Fe^{3+}-AA. Note: Fe^{3+}-AA is amber because of the presence of iron. The Ca^{2+}-AA is translucent but appears gray in these pictures because the films are sitting on a black background.

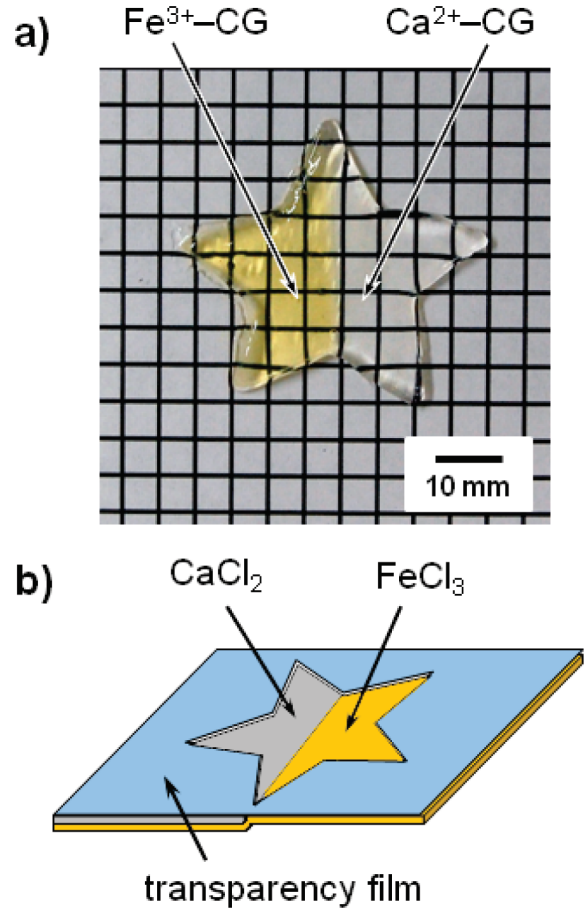

FIGURE 3. (a) Single hydrogel film of ι-CG in the shape of a star. The left half (pale yellow) is Fe^{3+}-CG, and the right half (clear) is Ca^{2+}-CG. (b) Schematic of the template used to produce this film.

Production of Gels with Step Gradients in the Concentration of Cross-Linking Ions. We constructed a template to vary the concentration of cross-linking ions across a hydrogel film by joining multiple pieces of paper wetted with different concentrations of the same cross-linking ion (Figure S2 in the Supporting Information). We cut

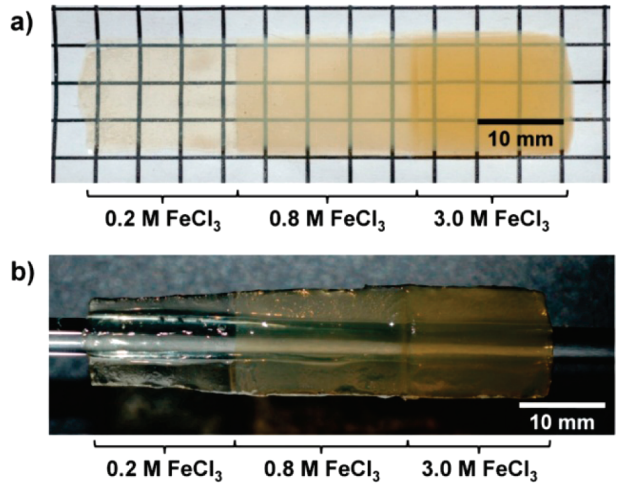

FIGURE 4. (a) Fe^{3+}-AA film containing a step gradient in the concentration of the cross-linking ion, Fe^{3+}. (b) Fe^{3+}-AA film draped over a glass pipet to demonstrate the variable rigidity of the sections as a function of the concentration of Fe^{3+} used to cross-link the AA. The section made from the least concentrated solution of Fe^{3+} had the weakest mechanical rigidity. The labeled concentrations correspond to the solutions used to wet the region of the paper template that formed each section of the gel.

sheets of toner-covered paper into shaped pieces, wet them with different concentrations of cross-linking ions, and placed them next to each other (with a slight overlap between adjacent pieces). The layer of toner sandwiched between the adjacent sheets prevented ionic solutions on the sheets from mixing. A continuous film that contained a step gradient of cross-linking agent formed when a solution of un-cross-linked polymer came in contact with the surface of the joined template.

For example, to make a film of Fe^{3+}-AA with a spatial step gradient in the cross-linking density (Figure 4), we cut a piece of paper with a solid layer of toner on one side into rectangles. We wet each piece with a different solution of $FeCl_3$ (0.2, 0.8, and 3.0 M), placed the pieces next to each other with a small (~2 mm) amount of overlap, and secured them to a flat surface with tape. A 2% solution of AA poured onto this template formed a single film, which we removed from the paper with a spatula. The color of the gel increased from light orange to dark orange as the cross-linking density increased. The thicknesses of the regions of the film were 0.18 ± 0.02, 0.61 ± 0.03, and 0.90 ± 0.10 mm for the sections formed from 0.2, 0.8, and 3.0 M $FeCl_3$, respectively.

The mechanical properties of the film were a function of the concentration of the solution used to cross-link each region. Figure 4b shows the film placed on a glass pipet. The section of the film cross-linked with the most dilute solution of Fe^{3+} was mechanically the weakest and conformed slightly to the shape of the pipet. Conversely, the section of the film cross-linked with the most concentrated solution of Fe^{3+} was the strongest and resisted bending.

Patterning Magnetic Regions into Hydrogel Films. When paramagnetic cations such as Ho^{3+} or Gd^{3+} are used to cross-link anionic polymers, the resulting ionotropic hydrogels respond to gradients in the magnetic field. The method described in Figure 1 can produce shaped regions of paramagnetic hydrogels within a film that allow the film to be oriented in a particular direction with a simple bar magnet. As a demonstration of this idea, we produced a film of Ca^{2+}-AA in the shape of an arrow with a strip of Ho^{3+}-AA inserted along its center (Figure S3 in the Supporting Information). When the film was placed in a Petri dish with water and agitated gently, the long axis of the strip aligned with the long axis of a bar magnet positioned under the dish. The incorporation of paramagnetic regions in films of ionotropic hydrogels makes it possible to manipulate films without the need for touching them.

Patterning Bacteria within a Hydrogel Film. Gel-in-gel structures made by these methods can also pattern bacteria within a hydrogel film. We selected *E. coli* as a model bacterium for study and transformed the plasmid pUC18 into competent *E. coli* BL21gold(DE3). This plasmid contains genes that encode for β-lactamase, which confers resistance to the antibiotic ampicillin, and β-galactosidase, which is overexpressed by the bacteria when induced by the addition of isopropyl β-D-1-thiogalactopyranoside (IPTG). β-Galactosidase metabolizes 5-bromo-4-chloro-3-indolyl-β-D-galactopyranoside (X-gal) to form a blue pigment. We included IPTG and X-gal in the solutions used to make the alginate films and used the formation of the blue pigment as a colorimetric tool to monitor the viability of bacterial colonies within the film. We made the films from a 2% solution of AA in lysogeny broth (LB) instead of deionized water to provide nutrients for the bacteria.

We began by testing the viability of *E. coli* in homogeneous alginate films cross-linked by 2 M solutions of different metals. The bacteria survived in Ca^{2+}-AA and Ba^{2+}-AA but not in Ni^{2+}-AA, Cu^{2+}-AA, Al^{3+}-AA, Gd^{3+}-AA, or Ho^{3+}-AA. These results are consistent with metal toxicity data reported for biofilms of *E. coli* JM109 (36). The Ni^{2+}-AA, Cu^{2+}-AA, and Al^{3+}-AA films were mechanically weak and tore easily when handled. To produce films that were mechanically strong, we used solutions of cross-linking ions that were 1 M in $CaCl_2$ and 1 M in one of the toxic metals. These films could be manipulated without tearing, yet they were still toxic to *E. coli*.

Figure 5 depicts the culture of *E. coli* in a heterogeneous hexagon-shaped film of Ca^{2+}-AA with a central square-shaped region of Al^{3+}-AA + Ca^{2+}-AA. After incubation for 6 h at 37 °C, the bacteria grew almost exclusively in the region of gel cross-linked by Ca ions, as indicated by the formation of blue pigment in the outer region of the hexagon. The Al ions in the central region of the film inhibited the growth of bacteria, and the square region remained uncolored.

The resolution of the bacterial patterns can be limited by the affinity of the alginate for the toxic metal. The affinity of AA for metals, as measured by the R_f value for the ions traveling through a stationary phase of AA, roughly follows the order $Pb^{2+} > Ba^{2+} > Fe^{3+}$, $Al^{3+} > Cu^{2+}$, Cd^{2+}, $Ho^{3+} > Ca^{2+} > Zn^{2+}$, Co^{2+}, $Ni^{2+} > Mn^{2+}$, Mg^{2+} (37−40). For metals with low affinities, the ions can spread away from their original regions of gel and poison bacteria in regions intended to

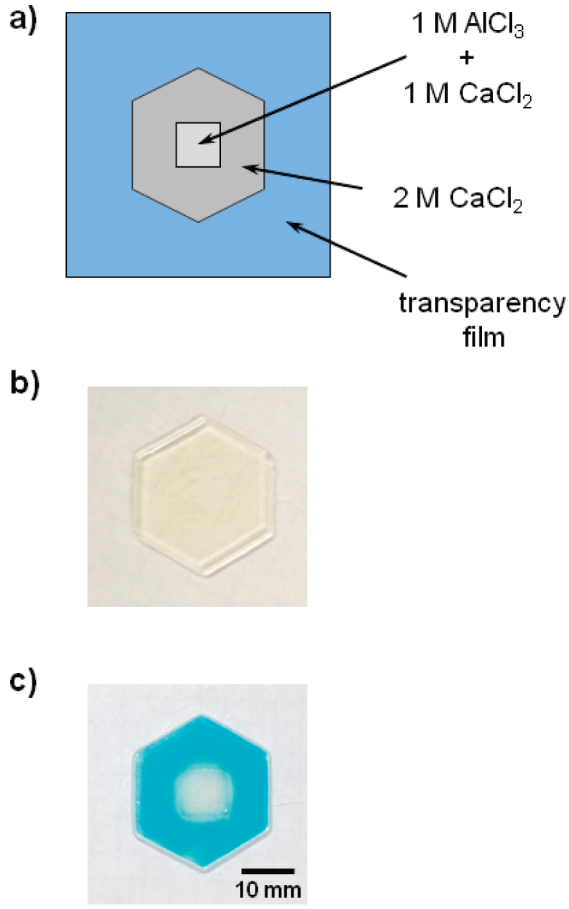

FIGURE 5. (a) Top view of a three-layered template used to form a hexagon-shaped film of Ca^{2+}-AA with a square region of AA cross-linked with a mixture of Al^{3+} and Ca^{2+}. (b) Heterogeneous film of AA with LB, IPTG, X-gal, and *E. coli* formed from the template above. (c) Same film after 6 h of incubation at 37 °C. The blue color arises from the metabolism of X-gal by β-galactosidase (expressed by *E. coli*) and indicates the presence of viable colonies of the bacteria. Ca^{2+}-AA is nontoxic to the cells, which survive in the outer region of the film (blue). Colonies do not grow in the central region (clear) because of the toxicity of Al^{3+} to the bacteria.

have viable colonies. Metals with high affinities for AA (e.g., Al^{3+}) tended not to spread around the film: a desired pattern could be produced with greater spatial fidelity.

CONCLUSIONS

These techniques provide a method for the generation of heterogeneous films of ionotropic hydrogels. The method can be used to pattern one hydrogel inside another ("gel-in-gel" structures) or to make hydrogels that contain step gradients in the concentration of the cross-linking ion. The fabrication of heterogeneous ionotropic gels is simple and occurs under mild conditions (in aqueous solutions at room temperature with no UV light). We can produce films with feature sizes down to several millimeters, which we believe is satisfactory for many biological and medical applications. While the method uses a laser cutter to pattern the templates, this specialized device can be replaced by other paper cutting devices (e.g., a razor blade or hole puncher) for the fabrication of ordinary shapes.

We demonstrated that we could control the growth of *E. coli* in films of alginate by patterning ions that inhibited or supported growth of the bacterium. For best results, the polymer should have a high affinity for the ions used as cross-linking agents to minimize the diffusion of toxic ions throughout the film. The technique described here represents a new method for patterning and controlling the growth of microorganisms in hydrogels to study biofilms and interactions in and among microbial communities.

Acknowledgment. This work was supported by the BASF Advanced Research Initiative and the National Institutes of Health (NIH; Award EHS-R01 ES016665). P.J.B. is grateful for graduate fellowships from the National Science Foundation and the Harvard Origins-of-Life Initiative. M.G. acknowledges an NSEC fellowship under NSF Award PHY-0646094. E.T.M. thanks the NIH for a postdoctoral fellowship (Grant NRSA-GM076971).

Supporting Information Available: Experimental procedures for the construction of templates, preparation of heterogeneous films, and patterning of bacteria within the films. This material is available free of charge via the Internet at http://pubs.acs.org.

REFERENCES AND NOTES

(1) Augst, A. D.; Kong, H. J.; Mooney, D. J. *Macromol. Biosci.* **2006**, *6*, 623–633.
(2) Michel, A.-S.; Mestdagh, M. M.; Axelos, M. A. V. *Int. J. Biol. Macromol.* **1997**, *21*, 195–200.
(3) Rinaudo, M. *Polym. Int.* **2008**, *57*, 397–430.
(4) Peppas, N. A.; Hilt, J. Z.; Khademhosseini, A.; Langer, R. *Adv. Mater.* **2006**, *18*, 1345–1360.
(5) Smidsrød, O.; Skjåk-Bræk, G. *Trends Biotechnol.* **1990**, *8*, 71–78.
(6) Tønnesen, H. H.; Karlsen, J. *Drug Dev. Ind. Pharm.* **2002**, *28*, 621–630.
(7) Gombotz, W. R.; Wee, S. F. *Adv. Drug Delivery Rev.* **1998**, *31*, 267–285.
(8) Wong, M. In *Biopolymer Methods in Tissue Engineering*; Hollander, A. P., Hatton, P. V., Eds.; Humana Press, Inc.: Totowa, NJ, 2003; Vol. 238, pp 77—86.
(9) Wang, X.; Haasch, R. T.; Bohn, P. W. *Langmuir* **2005**, *21*, 8452–8459.
(10) Alon, U.; Surette, M. G.; Barkai, N.; Leibler, S. *Nature* **1999**, *397*, 168–171.
(11) Devreotes, P. N.; Zigmond, S. H. *Annu. Rev. Cell Biol.* **1988**, *4*, 649–686.
(12) Ni, Y.; Chiang, M. Y. M. *Soft Matter* **2007**, *3*, 1285–1292.
(13) Wong, J. Y.; Velasco, A.; Rajagopalan, P.; Pham, Q. *Langmuir* **2003**, *19*, 1908–1913.
(14) Liu, V. A.; Bhatia, S. N. *Biomed. Microdevices* **2002**, *4*, 257–266.
(15) Zguris, J. C.; Itle, L. J.; Koh, W.-G.; Pishko, M. V. *Langmuir* **2005**, *21*, 4168–4174.
(16) Burdick, J. A.; Khademhosseini, A.; Langer, R. *Langmuir* **2004**, *20*, 5153–5156.
(17) Zaari, N.; Rajagopalan, P.; Kim, S. K.; Engler, A. J.; Wong, J. Y. *Adv. Mater.* **2004**, *16*, 2133–2137.
(18) Klajn, R.; Fialkowski, M.; Bensemann, I. T.; Bitner, A.; Campbell, C. J.; Bishop, K.; Smoukov, S.; Grzybowski, B. A. *Nat. Mater.* **2004**, *3*, 729–735.
(19) Cohen, D. L.; Malone, E.; Lipson, H.; Bonassar, L. J. *Tissue Eng.* **2006**, *12*, 1325–1335.
(20) Costerton, J. W.; Stewart, P. S.; Greenberg, E. P. *Science* **1999**, *284*, 1318–1322.
(21) Hall-Stoodley, L.; Costerton, J. W.; Stoodley, P. *Nat. Rev. Microbiol.* **2004**, *2*, 95–108.
(22) Heilmann, C.; Schweitzer, O.; Gerke, C.; Vanittanakom, N.; Mack, D.; Götz, F. *Mol. Microbiol.* **1996**, *20*, 1083–1091.
(23) Beech, I. B.; Sunner, J. *Curr. Opin. Biotechnol.* **2004**, *15*, 181–186.
(24) Donlan, R. M.; Pipes, W. O.; Yohe, T. L. *Water Res.* **1994**, *28*, 1497–1503.
(25) Heilmann, C.; Hussain, M.; Peters, G.; Götz, F. *Mol. Microbiol.* **1997**, *24*, 1013–1024.

(26) Donlan, R. M. *Clin. Infect. Dis.* **2001**, *33*, 1387–1392.
(27) Donlan, R. M.; Costerton, J. W. *Clin. Microbiol. Rev.* **2002**, *15*, 167–193.
(28) Habash, M.; Reid, G. *J. Clin. Pharmacol.* **1999**, *39*, 887–898.
(29) DiTizio, V.; Ferguson, G. W.; Mittelman, M. W.; Khoury, A. E.; Bruce, A. W.; DiCosmo, F. *Biomaterials* **1998**, *19*, 1877–1884.
(30) Gopinathan, U.; Stapleton, F.; Sharma, S.; Willcox, M. D. P.; Sweeney, D. F.; Rao, G. N.; Holden, B. A. *J. Appl. Microbiol.* **1997**, *82*, 653–658.
(31) Shimizu, K.; Kobayakawa, S.; Tsuji, A.; Tochikubo, T. *Curr. Eye Res.* **2006**, *31*, 989–997.
(32) Bracher, P. J.; Gupta, M.; Whitesides, G. M. *Adv. Mater.* **2009**, *21*, 445–450.
(33) Martinez, A. W.; Phillips, S. T.; Butte, M. J.; Whitesides, G. M. *Angew. Chem., Int. Ed.* **2007**, *46*, 1318–1320.
(34) Martinez, A. W.; Phillips, S. T.; Carrilho, E.; Thomas, S. W., III; Sindi, H.; Whitesides, G. M. *Anal. Chem.* **2008**, *80*, 3699–3707.
(35) Xerox Material Safety Data Sheet, http://www.office.xerox.com/userdoc/P6250/6250_Web/pdfs/msds.pdf.
(36) Harrison, J. J.; Turner, R. J.; Ceri, H. *BMC Microbiol.* **2005**, *5*, 11.
(37) Cozzi, D.; Desideri, P. G.; Lepri, L.; Ciantelli, G. *J. Chromatogr.* **1968**, *35*, 396–404.
(38) Cozzi, D.; Desideri, P. G.; Lepri, L.; Ciantelli, G. *J. Chromatogr.* **1968**, *35*, 405–415.
(39) Muzzarelli, R. A. A. *Natural Chelating Polymers*; Pergamon Press: Oxford, U.K., 1973.
(40) Winkleman, A.; Bracher, P. J.; Gitlin, I.; Whitesides, G. M. *Chem. Mater.* **2007**, *19*, 1362–1368.

AM900340M