

Shaped Films of Ionotropic Hydrogels Fabricated Using Templates of Patterned Paper

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This article describes the use of paper—patterned either by hand or with a color laser printer—to fabricate films of ionotropic hydrogels structured into regular shapes with lateral dimensions from 2 mm to several centimeters, and with thicknesses from 0.2 to 1.3 mm. Water-soluble polymers such as alginic acid (AA) and carboxymethyl cellulose (CMC) form hydrogel films with defined shapes when solutions of these polymers are brought into contact with patterned templates of paper wetted with aqueous solutions of multivalent cations. The hydrogel films have sufficient mechanical strength to be handled with tweezers, and they retain their shapes when stored in water for weeks. When multivalent cations of high magnetic susceptibility (χ) cross-link the polymers (as in holmium- or gadolinium-cross-linked AA, Ho^{3+} -AA and Gd^{3+} -AA), the films can be manipulated using rare-earth bar magnets.

This procedure provides a new method for the fabrication of hydrogel films into structures that cannot be achieved (easily, or at all) using other methods. It renders possible the production of topographically and topologically complex three-dimensional shapes (e.g., interlocking rings and Möbius strips), and overcomes the difficulty presented in adding solutions of cross-linking ions to millimeter-sized layers of uncured polymer without deforming the shapes of the resulting hydrogel films. For certain patterns, our procedure provides a manufacturing method, since the patterned paper templates can be reused.

Ionotropic hydrogels are hydrated gel matrices that form when metal ions cross-link water-soluble linear polymers. Polymers that form hydrogels in the presence of multivalent cations include AA, CMC, ι -carrageenan (CG), poly(galacturonic acid) (PG), and poly[bis(4-carboxyphenoxy)-phosphazene].^[1] Ionotropic hydrogels shaped in three dimensions with controlled compositions are an important class of biomaterials, with applications in the fields of controlled (drug) delivery, cellular immobilization, and biomimicry.^[2] Ionotropic hydrogels can serve as delivery devices for drugs and proteins when the polymers are biocompatible and degrade slowly relative to conventional sustained/slow-release systems.^[3] Ionotropic hydrogels (especially Ca^{2+} -AA) have been investigated as cellular scaffolds, but usually as spherical beads because other 3D structures are more difficult to fabricate.^[4] Lim and Sun demonstrated that alginate gels are suitable materials for cell encapsulation, although cells will usually not attach directly to

unmodified alginate.^[5] Ionotropic hydrogels modified to present ligands for cell receptors have been used to mimic extracellular matrices (ECMs) that provide structural support to cells in tissue.^[3,6]

Biomimetic materials are synthetic materials that have physical properties and biological functions similar to materials found in nature. Hydrogels have attracted interest as biomimetic materials due to their pliability, extent of hydration, low toxicity, and biocompatibility. For instance, Aizenberg and coworkers used hydrogels to mimic the microlens arrays of brittle stars (*Ophiocoma wendii*).^[7] Hydrogels can also be used to study creatures that creep, crawl, inch, and slither. Mahadevan et al. agitated cylindrical rods of hydrogels to simulate the muscular contractions of a snail.^[8] Yeghiazarian et al. heated rods of thermosensitive hydrogels confined to glass capillaries to mimic the movement of earthworms.^[9] Other uses for ionotropic hydrogels include as binding agents in food products,^[10] as sorbents for toxic metals,^[11] and as wound dressings.^[12] AA cross-linked with radioactive Ho^{3+} has been used in anti-tumor therapy that can be monitored by MRI.^[13]

On sub-micrometer scales, Lahav et al. used microfluidic channels to pattern thin films of poly(acrylic acid) (PAA) cross-linked by metallic ions (e.g., Pb^{2+} , Ba^{2+} , Zn^{2+} , Pd^{2+} , Cu^{2+} , La^{3+} , Ho^{3+}).^[14] Winkleman et al. used photolithography to create patterns of thin films of PAA cross-linked by metal ions to assist in the production of low- κ dielectric materials.^[15] While our group and others have fabricated sub-micrometer-thick layers of metal cross-linked polymers such as PAA, films of ionotropic hydrogels with millimeter thicknesses are more challenging to construct, because of the difficulty of introducing the multivalent cations across the entire surface of the film without disturbing the uniformity of the uncured layer of polymer. Techniques used to construct ionotropic gels on this scale include that of Chang et al., who produced synthetic facial implants in the shapes of chins, cheeks, and nasal septa out of hydrogels by injecting mixtures of chondrocytes, alginate, and CaSO_4 into Silastic molds.^[16] Cohen et al. developed a robotic system to fabricate arbitrary 3D hydrogel structures by sequentially printing 2D layers of Ca^{2+} -AA patterned with 1.2 mm wide and 0.8 mm tall threads of a solution of alginate for which cross-linking with CaSO_4 was initiated prior to deposition.^[17] Liu and coworkers fabricated microscale spheres, rods, plugs, disks, and threads of alginate hydrogels using a flow-focusing microfluidic device.^[18] In their process, droplets of a solution of sodium alginate collided with droplets of a solution of CaCl_2 inside microchannels and formed hydrogels. Solutions of sodium alginate gelled into membranes when cast onto porous media—including paper—wetted with solutions of multivalent cations.^[19] Cheng and coworkers controlled the porosity of these

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membranes by adding paraffin and surfactants as emulsifying agents.^[20] Zimmermann et al. formed membranes of Ba^{2+} -AA by sandwiching a rectangular chamber (52 mm × 90 mm × 2 mm) filled with sodium alginate between two outer chambers that contained 20 mM BaCl_2 .^[21] The chambers were separated by cellulose filters that allowed the ionic solutions to diffuse into the inner chamber and cross-link the alginate over the course of 2–15 h.

The strategy we pursued for the production of shaped hydrogel films was to use a template to deliver the cations required for the formation of gels locally, in a pattern. An ideal template is easy-to-make, reusable, and able to be folded or bent into complex three-dimensional shapes. In this process, the template assumes a role that is analogous to the chrome mask in photolithography, or to the stamp in microcontact printing.

Paper is an ideal substrate to use as a template and reservoir for the construction of films of ionotropic hydrogels. Paper is thin and flat, and because it absorbs aqueous solutions of salts, it can store the multivalent cations used to cross-link polymers into hydrogels. Paper will not fracture when it is bent. This property allows strips and sheets of paper to be shaped into rings and other complex three-dimensional structures, which would be difficult to construct with other materials. Paper is also inexpensive, widely available, biocompatible, and easy to store, transport, and manipulate. We used Whatman No. 1 chromatography paper in this work. This paper is absorptive and sold commercially in sheets of convenient thickness. It is mechanically strong enough that it does not rip easily when handled, and thin enough that it does not jam the printer during production of templates. It also has a more uniform surface than other types of absorbent paper (e.g., paper towels), and proved effective in the construction of the templates described below.

In our previous work, we used photolithography to pattern solid designs of cross-linked epoxy throughout a sheet of paper.^[22]

Aqueous solutions wet the regions of exposed paper and did not diffuse into the regions of polymerized photoresist. The time-consuming process of photolithography was necessary because we needed to restrict the flow of aqueous solutions to confined channels. Here—to make shaped hydrogel films on a paper substrate—we needed only to control the shape of the *area* where the ionic solution met the solution of uncross-linked polymer. Consequently, the templates only required the presence of a two-dimensional hydrophobic barrier on one side of the paper instead of a three-dimensional hydrophobic barrier that permeated the entire sheet.

We found it possible to print effective paper templates using a standard color laser printer. After being heated to approximately 200 °C following printing, the toner used by these printers provided a barrier that prevented aqueous solutions present in the paper from coming into contact with the anionic polymer. We blocked the unpatterned side of the templates with waterproof tape to limit the formation of the hydrogel films to one side of the paper. For the production of more complex three-dimensional hydrogel structures, we patterned the paper by simply cutting it with scissors or with a paper cutter, followed by bending and pasting it into the desired template.

The affinity of AA for cations—as measured by the R_f values of the cations travelling through a stationary phase of AA—roughly follows the order: $\text{Pb}^{2+} > \text{Ba}^{2+} > \text{Fe}^{3+}$, $\text{Al}^{3+} > \text{Cu}^{2+}$, Cd^{2+} , $\text{Ho}^{3+} > \text{Ca}^{2+} > \text{Zn}^{2+}$, Co^{2+} , $\text{Ni}^{2+} > \text{Mn}^{2+}$, Mg^{2+} .^[23–26] For our initial experiments, we selected Fe^{3+} to cross-link the AA due to the high affinity of the polymer for this cation relative to most other metals. Iron(III) is also inexpensive and nontoxic. The capacity of the Whatman No. 1 chromatography paper for a solution of 1 M FeCl_3 was $10 \pm 1 \mu\text{L cm}^{-2}$ of paper; for 2 M FeCl_3 it was $11 \pm 1 \mu\text{L cm}^{-2}$; and for saturated FeCl_3 it was $12 \pm 3 \mu\text{L cm}^{-2}$. These values and the value for deionized water ($11 \pm 1 \mu\text{L cm}^{-2}$) matched within the error of the experiment. To fabricate hydrogel films that responded to a magnetic field, we selected Ho^{3+} and Gd^{3+} as the cross-linking cations. The magnetic responses of these materials arise from the paramagnetism of these ions. As an added benefit, the colors of Fe^{3+} and Ho^{3+} , yellow and pink, respectively, rendered tracking the movement of these ions from the paper into the gel easy to monitor by eye. We also demonstrated that films could be cross-linked with Ca^{2+} (for maximum biocompatibility) and Ba^{2+} (for potential application to X-ray imaging).

Figure 1 illustrates the two methods we used to produce the paper templates: i) printing sheets of templates with a color laser printer and ii) constructing templates by hand, by cutting, bending, folding, taping, and pasting pieces of paper. In the printing method, a pattern designed in a simple graphics-design program such as Microsoft PowerPoint was printed onto a sheet of paper using the settings that applied the most toner with the highest print quality. We printed each

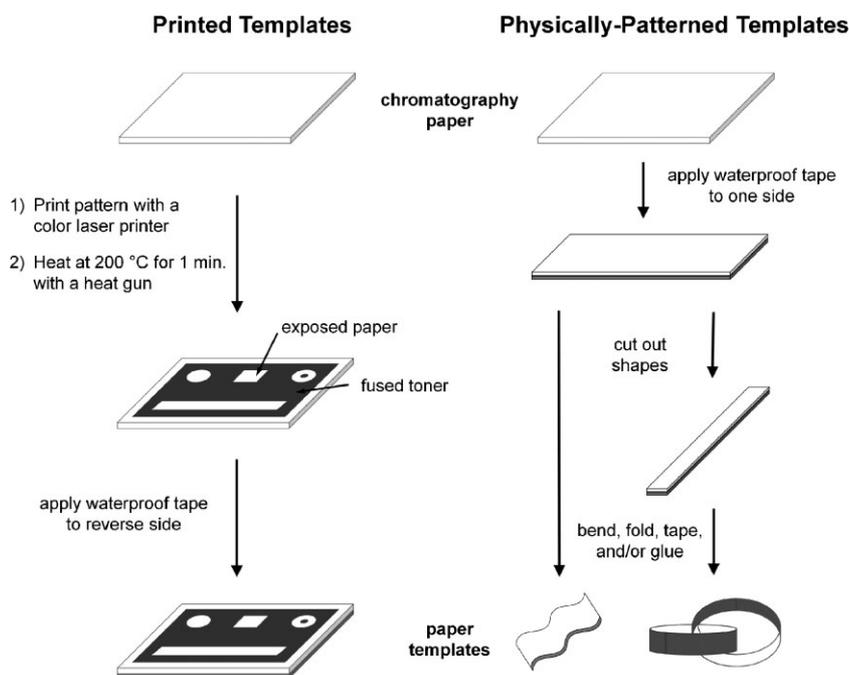


Figure 1. Schematic diagram showing the steps for the fabrication of the paper templates used to generate the shaped films of ionotropic hydrogels shown in this study.

image on the same sheet of paper three times, and heated the printouts at approximately 200 °C for 30 s with a heat gun to provide a hydrophobic barrier of sufficient thickness and uniformity to block the passage of aqueous solutions through the plastic toner. Ionic solutions placed on the regions not covered with toner wicked freely into the paper (including under the printed template).

We also used scissors, razor blades, and a paper cutter to cut paper into more complex three-dimensional shapes when folded, bent, and held into place with tape or adhesive. This second method allowed the production of complex structures such as “tall” 3D rings by joining the two ends of a strip of paper, and interlocking rings by joining the two ends of a second strip threaded through another ringed template. All of the paper templates were sealed on one side with waterproof tape to ensure that the ions leached into the polymer from only one side of the paper. The printed templates for making 2D films are reusable.

To produce the hydrogel films, we wet the templates described above with solutions containing multivalent metal ions, and the wetted templates were coated with or immersed in solutions of uncross-linked linear polymer. Figure 2 illustrates a specific example: the steps for creating 2D-shaped films of Fe^{3+} -AA using patterned paper. To begin, we wet the paper template with an aqueous solution of FeCl_3 (Fig. 2b). Once the ionic solution had wet the paper template uniformly, a 1.5% w/w solution of sodium alginate was applied to a glass slide that was then pressed against the paper template such that each shape was in contact with polymer (Fig. 2c). The margins of the slide had several layers of tape to serve as spacers between the paper and the glass, to control the thickness of the films. The ions leached out of the paper to form the hydrogel. After 3 min, we used a stream of deionized water to wash the excess (uncross-linked) polymer away from the films (Fig. 2d). The gentle prying action of a metal spatula released the films from the template (Fig. 2e).

The solutions of hydrogels used in this study (1.5% AA, 2% CMC, and 2% CG) were too viscous to wick into the chromatography paper. As a result, the gels formed *on* the paper and not *in* it. More dilute solutions (e.g., <0.5% AA), wet the paper but did not form robust gels.

The hydrogel films have sufficient mechanical strength that they can be handled with tweezers, but when not immersed in water, most films shrivel, harden, and dehydrate irreversibly. During formation of the films, the cross-linking ions diffuse from the template into the gel isotropically (Fig. 3). As a result, some of the cations cross-linked polymer outside of the vertical extension of the paper patterns, and the films were larger than the regions of exposed paper on the template. In a typical experiment—the formation of 10 mm wide disks of Fe^{3+} -AA from 1.5% AA and saturated FeCl_3 —the films had smooth edges that extended past the border of the template into the regions above the color toner by 0.88 ± 0.21 mm in all directions. Consistent with isotropic diffusion of the cross-linking ions, the thicknesses of these films were similar, with an average of 0.96 ± 0.14 mm.

For each film, the face that formed in contact with the template was uniformly flat. The “top” face could be left unconstrained such that it was flat in the center and tapered near the edges, or constrained by a top mold such that it was flat or textured (Fig. 3).

We used both the printer-based and physical methods to construct templates for the production of rings and other complex

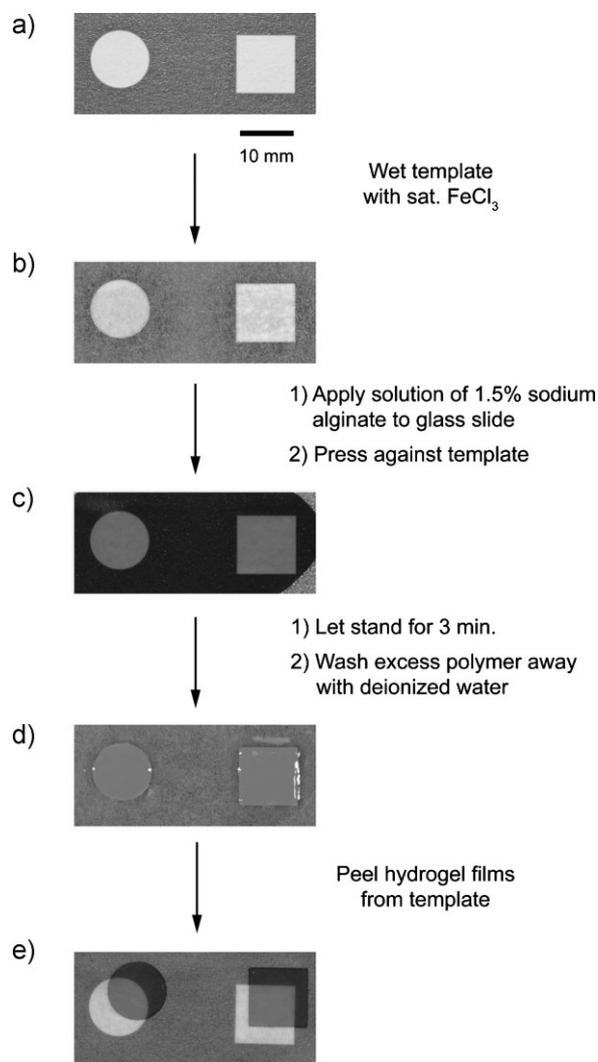


Figure 2. Schematic diagram that depicts the procedure for fabricating shaped films of Fe^{3+} -AA using a template of patterned paper. a) We printed a hydrophobic layer of toner onto chromatography paper with a color laser printer to form the templates. b) Next, we wet the paper with a saturated solution of FeCl_3 . c) A solution of sodium alginate containing a red dye (to improve contrast) was applied to a glass slide and the slide was pressed against the template. d) The hydrogel films formed on the template after the ions left the paper and cross-linked the polymer. e) Gently prying at the edges of the shapes with a spatula freed the films from the template. A color version of this figure appears as Figure S2 in the Supporting Information.

structures (Fig. 4). In the printer-based method, rings patterned onto the paper served as templates for the formation of hydrogel films in the process described above. In the physical method, we used a paper cutter to slice the chromatography paper into strips, which formed rings when bent and held closed with tape or glue. When wetted with solutions of metal salts, these templates formed isotropic hydrogels following immersion in solutions of uncross-linked polymer. After 1–2 min of immersion, the templates were removed and washed in a bath of water with gentle agitation. The paper could be peeled back from the hydrogel films by hand with the help of a metal spatula. For some of the more complex shapes, such as the interlocking rings,

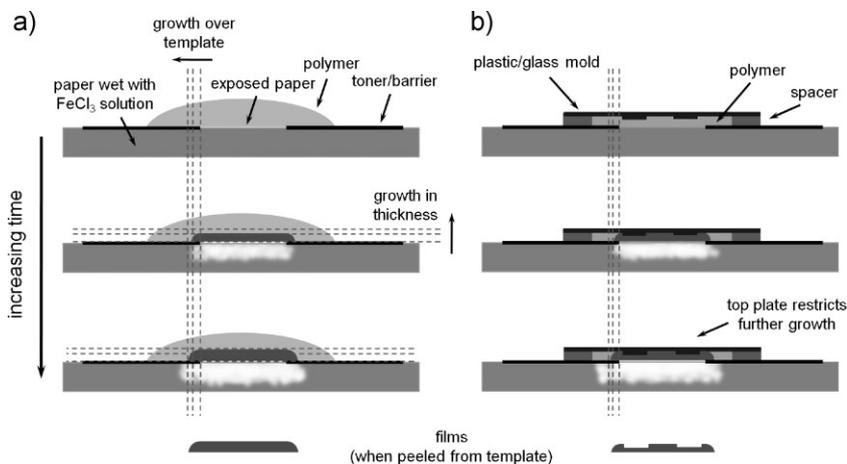


Figure 3. Schematic depicting the formation of hydrogel films on paper templates wetted with cross-linking agent. a) Preparation of a film through unconstrained growth. The ions spread isotropically from the exposed regions of paper into the uncross-linked aqueous solution of anionic polymer, such that the thickness of the film matched the distance the sides extended past the edge of the shaped template. b) Preparation of a film with a textured top surface using a molded plate. The plate controls the shape of the surface, and the spacer units (typically, layers of tape) control the thickness of the film. A color version of this figure appears as Figure S3 in the Supporting Information.

careful removal of the templates avoided fracturing of the gels. We found that films formed from mixtures of AA and CG (typically, 1% AA and 0.6% CG), fractured less often during removal of the template than those formed from AA alone. Iontropic hydrogels prepared by cross-linking α -CG with multivalent cations are typically soft and elastic.^[27] The molecular structure of Ca^{2+} -CG has been characterized by X-ray diffraction.^[28,29] Helices of the polymer chains—bridged by Ca^{2+} ions and water molecules—can slide past each other to form various polymorphs of the material.^[29] The addition of CG to polymers that form brittle ionotropic hydrogels has been used to increase the elasticity of the resulting gels.^[27] For the production of rings and interlocking rings, where the templates were held together with glue, soaking the structures in water loosened the adhesive such that the template could be peeled back from the gel from the seams.

In an attempt to make colored films, we used solutions of sodium alginate spiked with cationic dyes (e.g., Acridine orange) and anionic dyes (e.g., Cibacron Brilliant Red 3BA and Cibacron Brilliant Blue F3GA). The resulting films were colored when initially peeled from the template, but unless the dye precipitated in the presence of the cross-linking cation, the color disappeared after several hours when the films were stored in water. In contrast, the suspension of insoluble colored pigments in the solutions of polymer resulted in colorfast films upon gelation. When the solution of uncross-linked polymer contained a pigment (usually 2% w/w) such as carbon black or ferric oxide (Pigment Red 101), the colored particles became trapped in the hydrogel following the introduction of multivalent cations. The color did not fade noticeably while the pigmented films were stored in water.

When the polymers were cross-linked with Ho^{3+} or Gd^{3+} , the resulting films had a magnetic susceptibility that was high enough to adhere to a simple bar magnet. Films cross-linked with

Al^{3+} , Ca^{2+} , or Fe^{3+} did not adhere to the magnet (Fig. 5). Bar magnets could be used to separate Gd^{3+} -cross-linked films from those cross-linked with other ions (Fig. S6 of Supporting Information). A bar magnet placed close to—or in contact with—a mixture of hydrogel films selectively attracted the films of highest magnetic susceptibility. The magnet could then drag these paramagnetic films to a new location. The attractive force was weak enough that the films would detach by directing a stream of water at them or by shaking the magnet. We generated hydrogel films that exhibited strong responses to magnetic fields by suspending ferrimagnetic particles of strontium iron oxide in the solutions of uncross-linked polymer prior to gelation (Fig. S7 of Supporting Information).

The techniques described in this paper represent a method for the generation of three-dimensional films of ionotropic hydrogels in both simple and complex shapes. The paper templates are reusable, and their production simply requires having access to a standard color laser printer. For applications

where the movement of ions throughout a piece of paper need not be controlled, the method marks a substantial improvement in speed and simplicity over our previously described photolithographic procedure for patterning paper. Many of the shapes produced in this study, such as the Möbius strips, are topologically complex and we are aware of no simple alternatives for their production. While replica molding could be used to make some of these shapes, the technique is ill-suited for the construction of structures such as interlocking rings. Removal of the molds without damaging the delicate hydrogel films would be challenging and tedious. The straightforward simplicity offered by the paper templating method renders possible the swift production of shaped films of ionotropic hydrogels for their use in biomedical research and practical applications.

Experimental

Preparation of Paper Templates: Templates for the production of shaped films of hydrogels were designed as slides in Microsoft PowerPoint. The designs were printed using a Xerox Phaser color laser printer (Model 6250) on chromatography paper (20 cm \times 20 cm) fed into the printer manually through tray #1. Each pattern was printed onto the same sheet of paper three times, in order to ensure that the thickness of the layer of toner was sufficient to serve as a hydrophobic barrier. The template was heated with a heat gun set to high ($\sim 200^\circ\text{C}$) for 1 min to seal cracks and holes in the layer of toner. Failure to heat the templates resulted in porous layers of toner that allowed the ionic solutions to pass into the solutions of uncross-linked polymers. (We specifically emphasize our use of a color laser printer because the wax-like toner typically found in color laser printers makes satisfactory waterproof barriers, while the carbon-powder toner used in “standard” black-and-white laser printers does not. A simple test to determine whether a given printer will produce satisfactory templates is to print a 2 cm \times 2 cm solid square (in triplicate) onto a sheet of chromatography paper, heat the square with a heat gun (200°C for 1 min), then pipette a 50 μL drop of 1 M FeCl_3 (or any other colored aqueous

Hydrogel Structure Associated Template

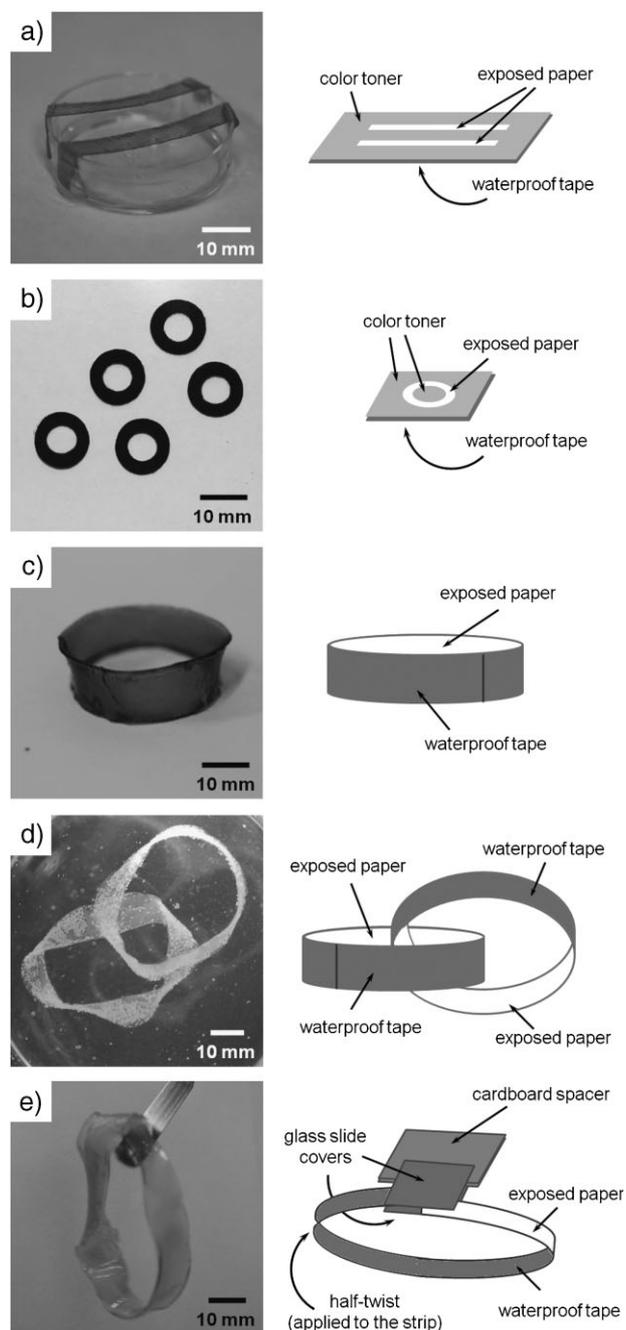


Figure 4. Hydrogel films patterned in various complex structures and schematic representations of the paper templates used to make them. a) Strips of Gd^{3+} -AA colored with Cibacron Brilliant Red 3BA suspended over a Petri dish (thickness = 0.70 ± 0.06 mm). b) "Short" 2D rings of Fe^{3+} -AA colored with activated carbon (thickness = 0.85 ± 0.03 mm). c) "Tall" 3D ring of Gd^{3+} -AA colored with Cibacron Brilliant Blue F3GA (thickness = 0.21 ± 0.06 mm). d) Interlocking "tall" rings of a mixture of 1% AA and 0.6% ι -CG cross-linked with Gd^{3+} (thickness = 0.44 ± 0.20 mm). e) A Möbius strip of 1% AA/0.6% CG cross-linked with Fe^{3+} (thickness = 0.90 ± 0.08 mm). A color version of this figure appears as Figure S4 in the Supporting Information.

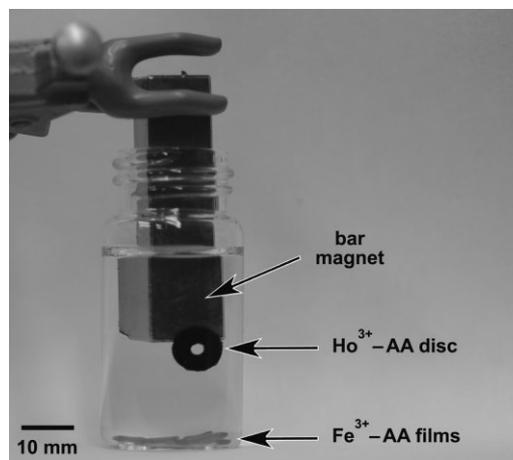


Figure 5. Photograph depicting the ability of a rare-earth bar magnet to selectively attract films of ionotropic hydrogels cross-linked with Ho^{3+} cations. The Ho^{3+} -cross-linked ring contains activated carbon (2% w/w) as a black pigment to distinguish it from the orange Fe^{3+} -cross-linked alginate films. A color version of this figure appears as Figure S5 in the Supporting Information.

solution) onto the square. If after 10 min the drop has not wet through the toner and into the paper, the templates produced on that printer will be satisfactory.) The templates were backed with a waterproof layer to prevent ions leaching from the back of the templates into the solutions of polymer. Transparent duct tape (3M Corporation, St. Paul, Minnesota) or self-adhesive Con-Tact Brand vinyl shelf liner (Kittrich Corporation, La Mirada, California) proved particularly effective for this purpose.

Preparation of Hydrogel Films from 2D Templates: The printed templates for the preparation of 2D films were taped to a flat surface (typically a polystyrene Petri dish), such that the paper would not curl when wet. The template was wet with a solution of cross-linking ions, and then an aliquot of polymer stock solution was poured or dispensed by pipette onto the template such that each shape was covered completely with a layer of polymer at least 2 mm thick. To construct films with flat tops, the polymer solution was applied to a glass slide before being pressed against the template with a suitable spacer material, to control the thickness of the film. For textured surfaces, the (flat) slide was replaced with a mold of plastic with the desired pattern. Following gelation, to avoid tearing the film, the mold was removed gently, perpendicular to the surface of the film (straight up). After the 1–3 min required for the gels to form, the excess uncross-linked polymer was washed away with copious amounts of water. The films could be freed from the paper with the gentle prying action of a metal spatula.

Preparation of Hydrogel Films from 3D Templates: To form rings, the ends of a strip of paper backed on one side with tape were joined by overlapping roughly 5–10 mm of the strip and fastening it with glue (UHU-brand glue stick). We found it easier to remove the paper following gelation when the ringed templates were constructed such that the back of the template (the taped side) faced outward. The template was wetted with ionic solution then immersed in a polymer solution. After 1 min, the structure was placed in a bath of water for 3–5 min and gently agitated to wash away excess uncross-linked polymer. The adhesive used to fuse the template loosened in the water and the template was gently peeled off of the hydrogel film with the aid of a metal spatula. Construction of the template for interlocking rings involved threading a second strip through a closed ring, then joining the ends of the second strip with glue. The templates for Möbius strips were made by gluing the ends of a strip to glass cover slides. Once the glue was dry, the strip was given a half-twist as the two glass cover slides were positioned in opposition to one another, as shown in Figure 4e. Cardboard served as a spacer material to hold the ends of the strip at a convenient distance apart during gelation.

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