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(54) Title: SHAPED FILMS OF HYDROGELS FABRICATED USING TEMPLATES OF PATTERNED PAPER

(57) **Abstract**: Methods of producing shaped hydrogels using templates of patterned substrates and uses for these hydrogels are described.

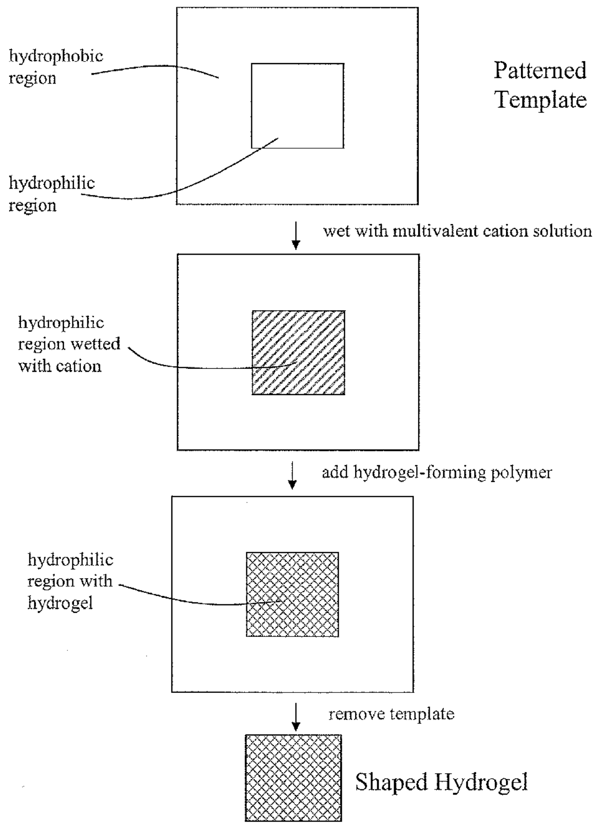

FIG. 1

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SHAPED FILMS OF HYDROGELS FABRICATED USING TEMPLATES OF PATTERNED PAPER

CROSS-REFERENCE TO RELATED APPLICATIONS

[0001] This application claims the benefit of U.S. Provisional Application No. 61/040,030, filed March 27, 2008, and U.S. Provisional Application No. 61/097,718, filed September 17, 2008, the contents of which are hereby incorporated in their entirety herein.

BACKGROUND

[0002] Hydrogels are three dimensional networks of polymers generally covalently or ionically cross-linked and exhibit the ability to swell in water and retain significant amounts of the absorbed water. Hydrogels may be made from a variety of compositions and some are classified as neutral, anionic and cationic hydrogels. Hydrogels can also be prepared by modifying existing polymers and comprise a broad class of polymeric materials which are insoluble, but significantly swollen by water (30-95% by wt).

[0003] Ionotropic hydrogels are a particular class of hydrogels that form when ions 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). Alginate cross-linked with Ca^{2+} (Ca^{2+}–AA) is non-toxic to many mammalian cells and can be used for tissue engineering (Augst et al., J. Macromol. Biosci. 6:623-633 (2006)), cell encapsulation (Smidsrød et al., Trends Biotechnol. 8:71-78 (1990)), controlled release of drugs (Tønnesen et al., J. Drug Dev. Ind. Pharm. 28:621–630(2002)), and wound dressings (Gombotz et al., Adv. Drug Deliv. Rev. 31:267-285(1998)).

[0004] The ability to design and fabricate composite materials such as films and functionalized surfaces is an important tool for research in cell biology and materials chemistry (Wang, et al., Langmuir 21:8452 (2005)). For example, the migration of bacteria and other cells can be controlled by gradients in the concentration of molecules (chemotaxis) (Alon et al., Nature 397:168-171(1999); Devreotes et al., Annu. Rev. Cell Biol. 4:649-686(1988)) or gradients in the mechanical rigidity of the substrate (durotaxis) (Ni et al., Soft Matter 3:1285-1292(2007); Wong et al., Langmuir 19:1908(2003)). Cellular interaction can be monitored using composite films that comprise patterned regions of distinct cells types within hydrogels (Liu et al., Biomed. Microdevices 4:257-266(2002); Zguris et al., Langmuir 21:4168-4174(2005)).

SUMMARY

[0005] In one aspect, the invention features a method of making a shaped hydrogel. The method comprises providing a template comprising a porous, hydrophilic region bounded by a fluid-impermeable barrier; contacting the porous, hydrophilic region of the template with an aqueous solution comprising a gelling agent, wherein the porous, hydrophilic region absorbs the solution; and contacting the porous, hydrophilic region of the template with a hydrogel precursor, wherein the gelling agent crosslinks the hydrogel precursor and forms a hydrogel. In one embodiment, the barrier substantially permeates the thickness of the porous, hydrophilic template.

[0006] In another aspect, the invention features a method of making a shaped composite hydrogel. The method comprises providing a first template comprising a porous, hydrophilic sheet and a fluid-impermeable layer disposed on the hydrophilic sheet, wherein said first template comprises a shape cut out of the template; providing a second template comprising a porous, hydrophilic sheet; contacting the porous, hydrophilic sheet of the first template with a first aqueous solution comprising a first gelling agent; contacting the porous, hydrophilic sheet of the second template with a second aqueous solution comprising a second gelling agent; contacting the first template on top of the second template such that the fluid-impermeable layer of the first template contacts the porous, hydrophilic sheet of the second template and at least a portion of the porous, hydrophilic sheet of the second template is exposed through the shape cut of the first template; and contacting the porous, hydrophilic sheet of the first template and the exposed portion of the porous, hydrophilic sheet of the second template with a hydrogel precursor, wherein the first gelling agent crosslinks the hydrogel precursor and forms a first type of hydrogel and the second gelling agent crosslinks the hydrogel precursor and forms a second type of hydrogel.

[0007] In another aspect, the invention features a method of making a shaped composite hydrogel. The method comprises providing a first template comprising a porous, hydrophilic sheet and a fluid-impermeable layer disposed on the hydrophilic sheet; wherein said first template comprises a shape cut out of the template; providing a second template comprising a porous, hydrophilic sheet; contacting the porous, hydrophilic sheet of the first template and the porous, hydrophilic sheet of the second template with an aqueous solution comprising a gelling agent; contacting the porous, hydrophilic sheet of the first template with a first hydrogel precursor, wherein the gelling agent crosslinks the first hydrogel precursor and forms a first hydrogel comprising a shape that is the same as the shape cut out of the template; contacting the first template comprising the first hydrogel with the second template such that the first hydrogel contacts the porous, hydrophilic sheet of the second template and at least a portion of the porous, hydrophilic sheet of the second template is exposed through the shape cut out of the fluid-impermeable layer of the first template; and contacting the exposed portion of the porous, hydrophilic sheet of the second template with a second hydrogel precursor, wherein the gelling agent from the porous, hydrophilic sheet of the second template crosslinks the second hydrogel precursor and forms a second hydrogel.

[0008] In another aspect, the invention features a method of making a composite hydrogel. The method comprises providing a first template comprising a porous, hydrophilic sheet and a fluid-impermeable layer disposed on the hydrophilic sheet; providing a second template comprising a porous, hydrophilic sheet and a fluid-impermeable layer disposed on the hydrophilic sheet; contacting the porous, hydrophilic sheet of the first template with a first solution comprising a first concentration of a gelling agent; contacting the porous, hydrophilic sheet of the second template with a second solution comprising a second concentration of the gelling agent; joining the first template and the second template end to end such that the porous, hydrophilic sheet of the first template and the porous, hydrophilic sheet of the second template form a continuous top face; and contacting the top face with a hydrogel precursor, wherein the gelling agent from the first template forms a first hydrogel having a first concentration of gelling agent, and the gelling agent from the second template forms a second hydrogel having a second concentration of gelling agent.

[0009] In any of the aspects described herein, the porous, hydrophilic substrate or sheet can comprise paper, nitrocellulose, cellulose acetate, cloth, or porous polymer film.

[0010] In any of the aspects described herein, the fluid-impermeable barrier or fluid-impermeable layer can comprise PDMS, poly(lactic-co-glycolic acid), epoxy, polystyrene, a polyether, a polyamide, PMMA, polycarbonate, polyethylene, polypropylene, a photoresist precursors, a wax or a fat.

[0011] In any of the aspects described herein, the hydrogel precursor can be a poly(phosphazene), a poly(acrylic acid), a poly(methacrylic acid), a copolymer of acrylic acid and methacrylic acid, poly(vinyl acetate), a sulfonated polymer, alginic acid (AA), carboxymethylcellulose (CMC), ι-carrageenan, poly(galacturonic acid) (PG), poly(acrylic acid) (PAA), poly(bis(4-carboxyphenoxy)-phosphazene, poly(vinyl amines), poly(vinyl pyridine), poly(vinyl imidazole), or an imino substituted polyphosphazenes.

[0012] In any of the aspects described herein, the gelling agent can be a multivalent cation or a multivalent anion. In some embodiments, the gelling agent is Pb^{2+}, Ba^{2+}, Fe^{3+}, Al^{3+}, Cu^{2+}, Cd^{2+}, Ho^{3+}, Ca^{2+}, Zn^{2+}, Co^{2+}, Ni^{2+}, Mn^{2+}, or Mg^{2+}, Sr^{2+}, Gd^{3+}, Pb^{2+}, Ra^{2+}, Fe^{2+}, Pd^{2+}, Bi^{3+}, Hg^{2+}, Au^{3+}, Co^{2+}, Co^{3+}, Cr^{2+}, Cr^{3+}, Mn^{4+}, Pt^{2+}, Pt^{4+}, Sn^{2+}, Sn^{4+}, Ce^{3+}, Ce^{4+}, Ga^{3+}, V^{3+}, or Rh^{3+}.

[0013] In any of the aspects described herein, the hydrogel can be shaped into any form. For example, the hydrogel can be shaped into a circle, a square, a rectangle, a torus, a disc, or a Mobius strip.

BRIEF DESCRIPTION OF THE DRAWINGS

[0014] The foregoing and other objects of the present invention, the various features thereof, as well as the invention itself, may be more fully understood from the following description, when read together with the accompanying drawings, in which:

[0015] FIG. 1 is a schematic diagram illustrating steps for fabricating a shaped hydrogel using a patterned template.

[0016] FIG. 2A and FIG. 2B are schematic diagrams illustrating steps for fabricating paper templates used to generate shaped ionotropic hydrogels.

[0017] FIGS. 3A-3E are schematic illustrations that depict steps for making shaped hydrogels of Fe^{3+}–AA using a template of patterned paper.

[0018] FIG. 4A is a schematic diagram illustrating the formation of hydrogel films on paper templates wetted with cross-linking agent through unconstrained growth. FIG. 4B is schematic diagram illustrating the formation of hydrogel films on paper templates wetted with cross-linking agent using a molded top plate.

[0019] FIG. 5A is an image of strips of Gd^{3+}–AA hydrogels colored with Cibacron Brilliant Red 3BA suspended over a Petri dish and a schematic of the paper template used to make them. FIG. 5B is an image of "short" rings of Fe^{3+}–AA hydrogels colored with activated carbon and a schematic of the paper template used to make them. FIG. 5C is an image of a "tall" ring of Gd^{3+}–AA hydrogel colored with Cibacron Brilliant Blue F3GA and a schematic of the paper template used to make it. FIG. 5D is an image of interlocking "tall" rings of a mixture of 1% AA and 0.6% ι-carrageenan cross-linked with Gd^{3+} and a schematic of the paper templates used to make them. FIG. 5E is an image of a Möbius strip of 1% AA/0.6% CG cross-linked with Fe^{3+} and a schematic of the paper template used to make it.

[0020] FIG. 6 is an image of a bar magnet selectively attracting films of ionotropic hydrogels cross-linked with Ho^{3+} cations.

[0021] FIG. 7A is an image of an arrow-shaped hydrogel film with a central strip of Ho^{3+}–AA aligned with the long-axis of a bar magnet positioned beneath the container of the film. FIG. 7B is a schematic illustration of the template used to produce the arrow-shaped magnetic hydrogel film.

[0022] FIGS. 8A-8C are images depicting the separation of Ho^{3+}-cross-linked hydrogel films (black) from a mixture of alginate films.

[0023] FIG. 9 is an image of a hydrogel ribbon made from a mixture of 1% AA and 0.6% ι-carrageenan and containing micron-sized particles of strontium iron oxide, which was attracted to a bar magnet.

[0024] FIGS. 10A-10G are schematic illustrations that depict steps for fabricating composite ionotropic gels made with different cross-linking ions.

[0025] FIG. 11A is an image of an alginate film formed with shapes of Fe^{3+}–AA surrounded by Ca^{2+}–AA. FIG. 11B is an image of the "inverse" film containing shapes of Ca^{2+}–AA surrounded by Fe^{3+}–AA.

[0026] FIG. 12A is an image of a single hydrogel film of ι-carrageenan in the shape of a star, where the left half is Fe^{3+}–CG and the right half is Ca^{2+}–CG. FIG. 12B is a schematic of the template used to produce this film.

[0027] FIGS. 13A-13F are schematic illustrations that depict steps for fabricating composite films of ionotropic gels made with different solutions of polymers.

[0028] FIG. 14 is an image of a continuous film with circles of Fe^{3+}–AA colored with Cibacron Brilliant Blue F3GA (right) and Cibacron Brilliant Red 3BA (left) surrounded by an un-dyed Fe^{3+}–AA backround (light gray).

[0029] FIGS. 15A-15E are schematic illustrations that depict steps for fabricating gels with step-gradients in the concentration of cross-linking ions.

[0030] FIG. 16A is an image of a Fe^{3+}–AA hydrogel film containing a step-gradient in concentration of cross-linking ion, Fe^{3+}. FIG. 16B is an image of the Fe^{3+}–AA film from FIG. 16A placed on a glass pipette.

[0031] FIG. 17A is a schematic illustration 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+}. FIG. 17B is an image of a composite film of AA with LB, IPTG, X-gal, and *E. coli* formed from the template depicted in FIG. 17A. FIG. 17C is an image of the same film depicted in FIG. 17B after 6 hours of incubation at 37 °C.

DETAILED DESCRIPTION

[0032] All publications, patent applications, patents, and other references mentioned herein are incorporated by reference in their entirety. In addition, the materials, methods, and examples are illustrative only and not intended to be limiting. Unless otherwise defined, all technical and scientific terms used herein have the same meaning as commonly understood by one of ordinary skill in the art to which this invention belongs. Although methods and materials similar or equivalent to those described herein can be used in the practice or testing of the present invention, suitable methods and materials are described below.

I. Methods of Making Hydrogels

[0033] The methods described herein relate to the fabrication of hydrogels, e.g., shaped hydrogels, on patterned templates, e.g., patterned paper. Figure 1 illustrates an exemplary method of fabricating a shaped hydrogel using a patterned template. As shown in Figure 1, a patterned template is provided having at least a hydrophilic region and usually one or more hydrophobic regions. The shape of the hydrophilic region will dictate the final shape of the hydrogel. The patterned template is contacted with a solution containing a gelling agent, e.g., a multivalent cation. Upon contacting the solution, the hydrophilic region of the template absorbs or wicks the aqueous solution, and the gelling agent, e.g., cations, becomes embedded within the template.

[0034] After the template is contacted with the solution containing the gelling agent, e.g., cation, the template is contacted with a solution containing a hydrogel-forming polymer. Upon contacting the gelling agent, e.g., cation, within the hydrophilic region of the template, the polymer forms a hydrogel. The dimensions of the hydrogel are dictated by the shape of the hydrophilic region. After the hydrogel is formed, the template can be removed to result in a free-standing shaped hydrogel.

[0035] Figure 4A illustrates a method of producing a hydrogel 440. In Figure 4A, a patterned template 410 is provided having hydrophilic region 412 and hydrophobic regions 420. Hydrophilic region 412 includes a gelling agent embedded within hydrophilic region 412. A solution 430 including a hydrogel precursor is added to template 410, where solution 430 contacts template 410 at region 415. When the hydrogel precursor contacts the gelling agent at region 415, the hydrogel precursor forms hydrogel 440.

[0036] In Figure 4B, a hydrogel 445 is produced using template 410, spacer 425, and mold 450. Template 410 includes hydrophilic region 412 and hydrophobic regions 420. Hydrophilic region 412 includes a gelling agent embedded within region 412. A solution 430 including a hydrogel precursor is added to template 410, where solution 430 contacts template 410 at region 415. Mold 450 is placed on top of solution 430, contacting solution 430 and creating a fluid impermeable seal 428 between mold 450 and spacer 425. Mold 450 includes ridges 451 in contact with solution 430. When the hydrogel precursor contacts the gelling agent at region 415, the hydrogel precursor forms hydrogel 445, which includes grooves 452 that are the inverse of the shape of ridges 451.

Templates

[0037] In some embodiments, a template is provided to deliver the gelling agents required for the formation of hydrogels locally in a pattern. In some embodiments, the template is made of paper. Paper is thin and flat, and because it absorbs aqueous solutions of salts, it can serve as a reservoir for the release of the gelling agents used to cross-link polymers into hydrogels. Because paper does not fracture when it is bent, strips and sheets of paper can be shaped into rings and other complex three-dimensional structures that may be difficult to construct with other materials.

[0038] Generally, a porous, hydrophilic template includes hydrophilic regions bounded by a fluid-impermeable material, e.g., hydrophobic material, that substantially permeates the thickness of the template. The hydrophobic material defines a pattern of hydrophilic regions bounded by the hydrophobic material. The shape of the resulting hydrogel is determined by the pattern of hydrophilic regions.

[0039] Figure 5 illustrates various shaped hydrogels made using hydrophilic templates. In Figure 5A, template 515 includes hydrophilic regions 512 bounded by hydrophobic regions 511. The strips of hydrophilic regions 512 are defined by the boundaries between hydrophobic regions 511 and hydrophilic regions 512. Template 515 is backed by waterproof layer 513. Hydrophilic regions 512 include a gelling agent. When template 515 is contacted with a solution containing a hydrogel precursor, the gelling agent within hydrophilic regions 512 contacts the hydrogel precursor, resulting in the formation of hydrogel 510. The dimensions of the strips of hydrogel 510 are determined by the shape of hydrophilic regions 512.

[0040] In Figure 5B, template 525 includes disc-shaped hydrophilic region 522 bounded by hydrophobic regions 521. The dimensions of hydrophilic regions 522 are defined by the boundaries between hydrophobic regions 521 and hydrophilic regions 522. Template 525 is backed by waterproof layer 523. Hydrophilic regions 522 include a gelling agent. When template 525 is contacted with a solution containing a hydrogel precursor, the gelling agent within hydrophilic regions 522 contacts the hydrogel precursor, resulting in the formation of hydrogel discs 520. The dimensions of the hydrogel discs 520 are determined by the shape of hydrophilic regions 522.

[0041] In Figure 5C, "tall" ring-shaped template 535 includes hydrophilic region 532 on the inner face of template 535 and a waterproof backing 533 on the outer face of template 535. Hydrophilic region 532 include a gelling agent. When template 535 is contacted with a solution containing a hydrogel precursor, the gelling agent within hydrophilic region 532 contacts the hydrogel precursor, resulting in the formation of "tall" ring-shaped hydrogel 530. The dimensions of the hydrogel 530 are determined by the dimensions of the hydrophilic region 532.

[0042] In Figure 5D, template 545 include two interlocking rings. Each interlocking ring includes hydrophilic region 542 on the inner face of the ring and a waterproof backing 543 on the outer face of the ring. Hydrophilic region 542 include a gelling agent. When template 545 is contacted with a solution containing a hydrogel precursor, the gelling agent within hydrophilic region 542 contacts the hydrogel precursor, resulting in the formation of interlocking rings of hydrogel 540. The dimensions of hydrogel 540 are determined by the dimensions of the hydrophilic regions 542.

[0043] In Figure 5E, template 555 is a Mobius strip. Template 555 includes hydrophilic region 552 on the inner face of the strip and a waterproof backing 553 on the outer face of the strip. Hydrophilic region 552 include a gelling agent. When template 555 is contacted with a solution containing a hydrogel precursor, the gelling agent within hydrophilic region 552 contacts the hydrogel precursor, resulting in the formation of Mobius strip hydrogel 550. The dimensions of hydrogel 550 are determined by the dimensions of the hydrophilic regions 552.

[0044] In some embodiments, a paper template is prepared by cutting it with scissors, razor blades, a hole punch, or a paper cutter (such as an automated laser cutter). Complex three-dimensional templates can be made by bending or folding the cut paper and using tape or adhesive to hold it in the desired template.

[0045] Templates can be made of any material that wicks fluids by capillary action and that is compatible with a selected patterning method. Nonlimiting examples include nitrocellulose and cellulose acetate, cellulosic paper, filter paper, cloth, and porous polymer film.

[0046] Preferably, the template is made of paper. Paper is inexpensive, widely available, biocompatible, and easy to store, transport, and manipulate. Paper-based devices absorb aqueous solutions by capillary action, and therefore, do not require an external power source to be filled. Stable reagents, e.g., multivalent cations, that wick into the paper in aqueous solutions can be stored for use at a later time. One nonlimiting example of paper that can be used as a template is Whatman No. 1 chromatography paper. This paper is absorptive and available commercially in sheets of specific thickness.

Patterning Templates

[0047] Any known method of patterning templates into hydrophobic and hydrophilic regions can be used in the methods described herein. Such methods are described in, e.g., WO 2008/049083.

[0048] In some embodiments, shaped hydrogel films can be made on a paper substrate by controlling the shape of the area where the ionic solution meets the solution of uncross-linked polymer. In such instances, the templates can include a two-dimensional hydrophobic barrier on one side of the paper instead of a three-dimensional hydrophobic barrier that permeates the entire sheet.

[0049] In some embodiments, a template pattern is formed by micro-contact printing. In such instances, a “stamp” of defined pattern is “inked” with a polymer, and pressed onto and through the template, thus forming barriers of a defined pattern. Micro-contact printing is described in, e.g., U.S. Patent No. 6,180,239.

[0050] In other embodiments, two-dimensional hydrophobic barriers can be created by applying waterproof tape to the template. In yet other embodiments, hydrophobic barriers can be formed by printing a continuous layer of toner onto sheets of paper using a standard color laser printer. When heated to seal any cracks or holes, the toner (a mixture of polyester resin, pigments, wax, and amorphous silicas) can block the passage of aqueous solutions between adjacent layers of paper. The template can be backed with waterproof layers such as adhesive tape and patterns can be cut into the sheets of paper. In other embodiments, barriers can be constructed from hydrophobic materials such as transparency film applied or fused to a hydrophilic absorptive material, like paper.

[0051] Figure 2 illustrates two exemplary methods for producing 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.

[0052] In the printing method depicted in Figure 2A, a porous hydrophilic substrate, such a chromatography paper 200, is provided. A pattern of toner is then applied to the paper 200 using a standard color printer (not shown). In the printing method, a pattern can be designed using, e.g., a graphics-design program (such as Microsoft PowerPoint) and printed onto a sheet of paper, e.g., using printer settings that apply the most amount of toner with the highest print quality. The thickness of the hydrophobic barrier, e.g., toner, can be controlled by printing each pattern onto the same sheet of paper multiple times.

[0053] As depicted in Figure 2A, the patterned paper can be heated to temperatures sufficient to melt and fuse the print toner, sealing any cracks or holes that may exist in the printed toner. Exemplary temperatures are those above the glass transition temperatures of the toner polymers and below the char temperatures of the paper template. In one or more embodiments, temperatures of 175-250° C can be used. In one exemplary printing process, each image is printed on the same sheet of paper three times. In some embodiments, the printing is performed using a ruler or laser-guided device to properly align the sheet before each printing step. In some embodiments, the sheet is thenheated at 175-250° C for 1 minute 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.

[0054] As depicted in Figure 2A, the resultant template 210 includes regions of exposed paper 220 corresponding to the desired shape of the hydrogel structure and water impervious, repellant regions of fused toner 230. A waterproof layer 240 can be applied to the backside of the template before or after the printing step. The waterproof layer can mitigate or prevent ionic solutions from collecting or pooling under the printed template. Any hydrophobic or non-porous material can be used as a waterproof layer. For example, transparent duct tape (3M Corporation, St. Paul, Minnesota) or self-adhesive Con-Tact Brand vinyl shelf liner (Kittrich Corporation, La Mirada, California) can be used.

[0055] As depicted in Figure 2B, another method involves physically patterning templates. In this process, a porous hydrophilic substrate260 , such a chromatography paper, can be used. The paper templates can be sealed on one side with a waterproof layer 270 such as a sheet of waterproof tape to ensure that the ions leach into the polymer from only one side of the paper. Shapes, e.g., strips 280, can be cut out of the paper, which can then be bent or folded into particular shapes 290. The shapes can be held together using, e.g., adhesive or tape. Alternatively, shape 295 can be cut from the adhesive-backed paper sheets into a desired geometrical shape.

Hydrogels

[0056] Any known hydrogel can be used in the methods described herein. Hydrogel matrices are described, for example, in U.S. Patent No. 5,906,934; Lin et al., Advanced Drug Delivery Rev. 58: 1379-1408 (2006); and Jen et al., Biotechnology and Bioengineering 50: 357-364 (2000). Polymers that can form ionic or covalently crosslinked hydrogels that are malleable can be used in the methods described herein. A “hydrogel”, as used herein, is a substance formed when a polymer is cross-linked via covalent, ionic, or hydrogen bonds to create a three-dimensional open-lattice structure that entraps water molecules to form a gel. The polymer can be an organic polymer. The polymer can be a natural or synthetic polymer. As used herein, a “hydrogel precursor” is a polymer that can be cross-linked via covalent, ionic, or hydrogen bonds to form a hydrogel.

[0057] Examples of materials that can be used to form a hydrogel include polysaccharides such as alginate, polyphosphazines, and polyacrylates (which are crosslinked ionically) or block copolymers such as Pluronics™ or Tetronics™, polyethylene oxide-polypropylene glycol block copolymers (which are crosslinked by temperature or pH, respectively). Other materials include proteins such as fibrin, polymers such as polyvinylpyrrolidone, hyaluronic acid, and collagen. In general, these polymers are at least partially soluble in aqueous solutions, such as water, buffered salt solutions, or aqueous alcohol solutions, that have charged side groups, or a monovalent ionic salt thereof.

[0058] Nonlimiting examples of polymers with acidic side groups that can be reacted with cations include poly(phosphazenes), poly(acrylic acids), poly(methacrylic acids), copolymers of acrylic acid and methacrylic acid, poly(vinyl acetate), sulfonated polymers, such as sulfonated polystyrene. Other nonlimiting examples include, e.g., alginic acid (AA), carboxymethylcellulose (CMC), ι-carrageenan, poly(galacturonic acid) (PG), poly(acrylic acid) (PAA), and poly(bis(4-carboxyphenoxy)-phosphazene. Copolymers having acidic side groups formed by reaction of acrylic or methacrylic acid and vinyl ether monomers or polymers can also be used. Nonlimiting examples of acidic groups include carboxylic acid groups, sulfonic acid groups, halogenated alcohol groups, phenolic OH groups, and acidic OH groups.

[0059] Nonlimiting examples of polymers with basic side groups that can be reacted with anions include poly(vinyl amines), poly(vinyl pyridine), poly(vinyl imidazole), and some imino substituted polyphosphazenes. The ammonium or quaternary salt of the polymers can also be formed from the backbone nitrogens or pendant imino groups. Nonlimiting examples of basic side groups include amino and imino groups.

Gelling Agents

[0060] As used herein, a “gelling agent” is any agent that cross-links a hydrogel precursor to form a hydrogel. For example, a gelling agent can cross-link the hydrogel precursor via covalent, ionic, or hydrogen bonds to form a hydrogel.

[0061] For example, a water soluble polymer with charged side groups can be ionically crosslinked by reacting the polymer with an aqueous solution containing a gelling agent of opposite charge, e.g., a multivalent ion of the opposite charge. In some embodiments, the polymer has acidic side groups and the gelling agent is a multivalent cation. In other embodiments, the polymer has basic side groups and the gelling agent is a multivalent anion.

[0062] In some embodiments, the gelling agent is a cation, e.g., Pb^{2+}, Ba^{2+}, Fe^{3+}, Al^{3+}, Cu^{2+}, Cd^{2+}, Ho^{3+}, Ca^{2+}, Zn^{2+}, Co^{2+}, Ni^{2+}, Mn^{2+}, or Mg^{2+}, Sr^{2+}, Gd^{3+}, Pb^{2+}, Ra^{2+},

Fe^{2+}, Pd^{2+}, Bi^{3+}, Hg^{2+}, Au^{3+}, Co^{2+}, Co^{3+}, Cr^{2+}, Cr^{3+}, Mn^{4+}, Pt^{2+}, Pt^{4+}, Sn^{2+}, Sn^{4+}, Ce^{3+}, Ce^{4+}, Ga^{3+}, V^{3+}, or Rh^{3+}.

[0063] In other embodiments, the gelling agent is a paramagnetic ion, e.g., a paramagnetic cation. Nonlimiting examples of paramagnetic cations include Ho^{3+} and Gd^{3+}.

Hydrogel Additives

[0064] In some embodiments, additives with desired properties may be added to the water soluble polymer solution to impart desirable properties in the resultant hydrogel structure. For example, solutions of hydrogel-forming polymers can be spiked with cationic dyes (e.g., Acridine orange) and anionic dyes (e.g., Cibacron Brilliant Red 3BA and Cibacron Brilliant Blue F3GA), resulting in hydrogel films of particular colors. In other embodiments, a suspension of insoluble colored pigments can be added to the solutions of polymers. Such insoluble colored pigments are known in the art and include, for example, carbon black, Phthalocyanine Alpha Blue (Pigment Blue 15), and ferric oxide (Pigment Red 101).

II. Methods of Making Composite Hydrogels

[0065] In some embodiments, composite hydrogels can be fabricated using paper templates, such as paper templates described herein. As used herein, a “composite hydrogel” is a hydrogel having two or more different types of hydrogels. For example, a composite hydrogel two types of hydrogels made from the same hydrogel precursor but being crosslinked by different gelling agents (or the same gelling agent in different concentrations). A composite hydrogel can also include two types of hydrogels made from different hydrogel precursors crosslinked by the same gelling agent. Exemplary composite hydrogels are illustrated in Figures 10 and 14.

Composite Hydrogels Containing Different Cross-Linking Ions

[0066] Figure 10 depicts an exemplary method for producing a composite hydrogel 1080 using a hydrogel precursor cross-linked with different gelling agents. As depicted in Figure 10A, a sheet of Whatman No. 1 chromatography paper 1000 is printed on one side with a layer of color laser toner 1010 (Figure 10A), a laser cutter (not shown) is used to cut shapes 1020 into it. The resulting composite hydrogel will include a first type of hydrogel having shapes 1020 and a second type of hydrogel having a shape that, at least in part, is the inverse of shapes 1020. Paper 1000 is then turned over (Figure 10B). This patterned paper 1030 and a new, unpatterned sheet 1040 are wetted with different solutions of gelling agents (Figure 10C). For example, the sheet 1030 is wet using a first cationic solution 1035 and sheet 1040 is wet using a second cationic solution 1045. The wetted sheets 1030 and 1040 are then dried, and stacked to form a stacked assembly 1050 as shown in Figure 10D. A sheet of transparency film 1060 is patterned with a laser cutter and placed on top of the paper. Film 1060 has a pattern (e.g., border 1065) that will shape the perimeter of the composite hydrogel (Figure 10E). The template is affixed to the Petri dish (e.g., with tape 1070). A solution of hydrogel precursor (e.g., alginic acid 1075) is poured onto the template and allowed to gel into a single composite film (Figure 10F). The film is rinsed with water and peeled from the template (e.g., with a metal spatula) (Figure 10G), resulting in composite hydrogel 1080. Composite hydrogel 1080 includes shaped hydrogels 1085 formed from hydrogel precursor 1075 and second cationic solution 1045. Shaped hydrogels 1085 have the same shape as cut shapes 1020. Composite hydrogel 1080 also includes hydrogel region 1086 formed from hydrogel precursor 1075 and first cationic solution 1035. Hydrogel region 1086 has regions 1087 that include the inverse of shapes 1020. Hydrogel region 1086 also has perimeter 1088 defined by the shape of border 1065. This method can be extended to incorporate additional cations by cutting the bottom layer into several pieces and wetting each piece with a different ionic solution.

Composite Hydrogels Containing Different Hydrogel Precursors

[0067] Figure 13 depicts an exemplary process to produce a composite hydrogel 1380 using two different hydrogel precursors cross-linked with the same gelling agent. As depicted in Figure 13, a sheet of chromatography paper 1300 is printed on one side with a layer of laser toner 1310 and a laser cutter (not shown) is used to cut shapes 1320 into it (Figure 13B). The resulting composite hydrogel will include a first type of hydrogel having shapes 1320 and a second type of hydrogel having a shape that, at least in part, is the inverse of shapes 1320. A laser cutter (not shown) is used to cut shapes 1320 into a sheet of waxed paper 1360 (Figure 13B).

[0068] Paper 1300 is then turned over and wetted using a cationic solution (not shown), and a solution of hydrogel precursor 1075 is poured onto the hydrophilic face of paper 1300 (Figure 13C). This results in hydrogel 1381 being formed on the hydrophilic face of paper 1300. Paper 1300 is then rinsed and turned over (Figure 13D). Waxed paper 1360, paper 1300, and a paper 1390 wetted with the cationic solution are stacked to form stacked assembly 1350 (Figure 13E). A solution of hydrogel precursor 1376 and a solution of hydrogel precursor 1377 are poured onto stacked assembly 1350 (Figure 13E). Stacked assembly 1350 is rinsed and waxed paper 1360, paper 1300, and paper 1390 are peeled away (Figure 13F), resulting in composite hydrogel 1380.

[0069] Composite hydrogel 1380 includes shaped hydrogels 1378 and 1379 formed from hydrogel precursor 1376 and 1377, respectively. Shaped hydrogels 1378 and 1379 have the same shape as cut shapes 1320. Composite hydrogel 1380 also includes hydrogel region 1381 formed from hydrogel precursor 1375. Hydrogel region 1381 has regions 1387 that include the inverse of shapes 1320.

Production of Composite Hydrogels with Step-Gradients in the Concentration of Gelling Agents

[0070] In some embodiments, composite hydrogels can be produced that contain gradients in concentration of gelling agents. One exemplary process is depicted schematically in Figure 15. As shown in Figure 15A, paper 1500 having toner 1510 on one side is cut into several pieces 1560 and turned over (Figure 15B). Each piece 1560 was wetted with different concentrations 1520, 1521, 1522 of the same gelling agent (Figure 15C). Pieces 1560 are then placed next to each other (with a slight overlap 1525 between adjacent pieces) and a solution of hydrogel precursor 1570 is applied to surface of the joined template (Figure 15D). The pieces 1560 are then rinsed and peeled, resulting in composite hydrogel 1580. Composite hydrogel 1580 includes hydrogel regions 1581, 1582, and 1583 that differ in the amount of gelling agent.

III. Methods of using Hydrogels

[0071] The shaped hydrogels can be used in many different applications. For example, the shaped hydrogels described herein can be used for controlled delivery of a compound of interest (e.g., a drug), cellular immobilization, and biomimicry. Biologically-compatible hydrogels can be nontoxic and can degrade slowly, and thus can be used as delivery devices for drugs and proteins. Shaped hydrogels, especially three-dimensional hydrogels, can also be used as cellular scaffolds (see, e.g., Lim et al., J Pharm Sci. 70:351-354 (1981)). Shaped hydrogels can also be modified to present ligands for cell receptors to model extracellular matrices (ECMs).

[0072] The shaped hydrogels described herein can also be used as biomimetic materials, due to their pliability, extent of hydration, low toxicity, and biocompatibility. Biomimetic materials are synthetic materials that have physical properties and biological functions that are similar to naturally-occurring biological materials. Hydrogels have been used to mimic the microlens arrays of brittlestars (see, e.g., Aizenberg et al., Nature 412:819-22 (2001)) and as models to study the movements of organisms (see, e.g., Mahadevan et al., Proc Natl Acad Sci U S A. 101:23-26 (2004)).

[0073] In some embodiments, the shaped hydrogels can contract, expand, and bend in response to stimuli such as exposure to an electric field, irradiation by light, and changes in pH or temperature. Other uses for shaped hydrogels include as binding agents in food products, as sorbents for toxic metals, and as wound dressings. In other embodiments, shaped hydrogels can be used in anti-tumor therapy, e.g., using radioactive cations to crosslink a hydrogel-forming polymer (see, e.g., Zielhuis et al., J. Biomed. Mater. Res. A. 82:892-898 (2007)).

[0074] In other embodiments, shaped hydrogels can be used to study biofilms. Biofilms are structurally complex communities of bacterial cells surrounded by hydrated extracellular polymeric matrices (Hall-Stoodley et al., Nat. Rev. Microbiol. 2:95-108 (2004); Costerton, Int. J. Antimicrob. Agents 11:217-221 (1999)). The formation of biofilms can occur on (and pose problems to) a variety of surfaces, including metals (Beech et al., Curr. Opin. Biotechnol. 15:181-186 (2004)), glass (Heilmann et al., Mol. Microbiol. 20:1083-1091 (1996)), plastics (Heilmann et al., Mol. Microbiol. 24:1013-1024 , (1997)), urinary tracts (Anderson et al., Science 301:105-107 (2003)), and implanted medical devices such as venous catheters, urinary catheters, contact lenses, prosthetic joints, prosthetic heart valves, and pacemakers (Habash et al., J. Clin. Pharmacol. 39:887-898 (1999); Donlan, Emerg. Infect. Dis. 7:277-281 (2001); Donlan, Emerg. Infect. Dis. 8:881-890 (2002)).

[0075] Bacterial adherence is the first stage in biofilm formation (Hall-Stoodley et al., Nat. Rev. Microbiol. 2:95-108 (2004)). The shaped hydrogels can be used to screen for agents that can inhibit biofilm formation (e.g., that inhibit bacterial adherence). For example, bacteria can be grown in a patterned composite hydrogel described herein that includes different test agents in different areas of the hydrogel. The ability of the bacteria to grow in different regions can be used to identify antibacterial agents.

[0076] In other embodiments, the shaped hydrogels can be used as coatings for medical devices. For example, the shaped hydrogels can include agents to inhibit bacterial growth (e.g., antibiotics) and used to coat medical instruments (see, e.g., DiTizio et al., Biomaterials 19:1877-1884 (1998); Gopinathan et al., J. Appl. Microbiol. 82:653-658, (1997); Shimizu et al., Curr. Eye Res. 31:989-997 (2006); and Barbucci et al., J. Mater. Chem. 12:3084 (2002)).

[0077] The invention is further illustrated by the following examples. The examples are provided for illustrative purposes only. They are not to be construed as limiting the scope or content of the invention in any way.

EXAMPLES

Example 1 – Methods of Producing Shaped Hydrogels

Reagents and Equipment

[0078] Sodium alginate, ι-carrageenan, the supplies for protein expression, and the inorganic salts used to cross-link the gels were purchased from Sigma-Aldrich (Milwaukee, Wisconsin) or VWR International (West Chester, Pennsylvania) unless otherwise noted. These materials were used without further purification. Solutions of sodium alginate (2% w/w), calcium chloride, calcium nitrate, ferric chloride, holmium nitrate pentahydrate, gadolinium chloride, copper nitrate, nickel nitrate, and barium chloride were made from 18 MΩ·cm deionized water. Whatman No. 1 chromatography paper was obtained from Sigma-Aldrich in square sheets measuring

20 x 20 cm. Sheets of transparency film were obtained from 3M (Item #CG3460) or OfficeMax (Itasca, Illinois, Item #OM96386).

[0079] A Xerox Phaser color laser printer (Model 6250) was used to print patterns of toner onto chromatography paper to form the templates. Each pattern was printed onto the same sheet of paper three times and the sheet was heated with a heat gun at ~200 °C for 30 seconds to seal cracks and holes in the layer of toner. In some instances, packaging tape (Staples® Clear View packaging tape) was applied to the paper as a hydrophobic barrier. Adobe Illustrator was used to draw patterns, which were imported to a laser cutter (Versa Laser cutter model VLS3.50). In the settings menu of the associated software package, the type of material was selected as "plastic (microsurface–deep engraving)" and the thickness of the material was entered as 1.4 mm (a value much greater than the actual thickness of the paper) to ensure that the desired cuts penetrated the entire sheet. The laser settings were set to 0% raster, 0% vector marking, 0% vector marking (on a scale from −50% to 50%). These settings were sufficient for all of the sheets we patterned with the laser cutter: chromatography paper with toner, chromatography paper with tape, and transparency film. The thicknesses of the hydrogel films were measured with a pair of digital calipers; the final values are reported as 90% confidence intervals based on the average of three trials.

Fabrication of Shaped Hydrogels

[0080] As depicted in Figure 3, Fe^{3+}-AA shaped hydrogels were fabricated. In a first step (a), the paper template 300 including cut shapes 310 was wet with an aqueous solution of $FeCl_3$. Once the ionic solution had wet the paper template 300 uniformly, in step (b) a 1.5% (w/w) solution of sodium alginate was applied to a glass slide that was then pressed against the paper template 300 such that each shape 310 was in contact with polymer (in step (c)). In some embodiments, a spacer was employed between the paper and the glass to control the thickness of the film. For the system depicted in Figure 3, the margins of the slide had several layers of tape to serve as a spacer between the paper and the glass. Upon contact, ions leached out of the paper to form the hydrogel. The assembly was allowed to stand for 3 minutes, after which excess polymer was removed by rinsing with water (step (d)). The polymer film was then released from the substrate by gently prying the hydrogels with a metal spatula (step (e)). The patterned paper template was reusable.

[0081] The solution of alginic acid used in this study (1.5% (w/w)) did not wick into the chromatography paper because the solution was too viscous. More dilute solutions of alginic acid (<0.5% by weight) wet the paper, but the polymers did not form robust gels at these concentrations.

[0082] The hydrogel films produced were sufficiently mechanically strong that they could be handled with tweezers. The hydrogels were stored in water. When not immersed in water, the hydrogel films sometimes shriveled, hardened, and dehydrated irreversibly.

[0083] Figure 5 depicts various complex shapes of hydrogel films that were produced. The production of rings and other complex structures was possible using both printer-based and physical methods to construct templates. In the printer-based method, shapes were patterned into the paper by printing toner onto the paper, as described above. In the physical patterning method, a paper cutter was used to slice the chromatography paper into strips, which were formed into rings and held closed with tape or glue. When wetted with solutions of metal salts, these templates formed ionotropic hydrogels following immersion in a solution of uncross-linked polymer. After 1 or 2 minutes of immersion, the films were removed and washed in a bath of water with gentle agitation. The paper templates could be removed by hand with the help of a metal spatula. For some of the more complex shapes, such as the interlocking rings, care was taken to avoid fracturing the gels during removal of the template. Films formed from a mixture of 1% AA and 0.6% (w/w) ι-carrageenan fractured less often during removal of the template than those formed from 1.5% AA.

Example 2 – Methods of Producing Shaped hydrogels Responsive to Magnetic Fields

[0084] Shaped hydrogels were formed that were capable of responding to magnetic fields. The incorporation of paramagnetic regions into films of ionotropic hydrogels allowed the hydrogels to be manipulated without touching them. When the hydrogel-forming polymers were cross-linked with Ho^{3+} or Gd^{3+} cations, the resulting hydrogel films exhibited a magnetic susceptibility that was high enough to adhere the hydrogel to a simple bar magnet, as illustrated in Figure 6 (the Ho^{3+}-crosslinked ring contained 2% activated carbon by weight). Films cross-linked with Al^{3+}, Ca^{2+}, or Fe^{3+} did not adhere to the magnet.

[0085] In another example, a film of Ca^{2+}–AA in the shape of an arrow with a strip of Ho^{3+}–AA inserted along its center was produced (Figure 7). An anionic red dye, Allura Red AC, was added to the solution of the sodium alginate used to form the film to improve the contrast. 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.

[0086] Bar magnets could also be used to separate Ho^{3+}-cross-linked films from those cross-linked with other ions. As depicted in Figure 8A, a starting mixture of Ho^{3+}-cross-linked alginate films (black) and Fe^{3+}-cross-linked films of various shapes (rings, discs, squares) were produced. The mixture shifted to one side of the Petri dish when the dish was tilted (Figure 8B). When a bar magnet was positioned near the mixture of films, the Ho^{3+}–AA shapes were selectively attracted and dragged to the opposite side of the dish (Figure 8C). Shaking the magnet released the films. By repeating this process, all of the Ho^{3+}-cross-linked films were completely separated from the initial mixture.

[0087] In another example, hydrogel films were also generated that exhibited strong responses to magnetic fields by suspending ferromagnetic particles of strontium iron oxide in the solutions of uncross-linked polymer prior to gelation. As shown in Figure 9, a ribbon of ferromagnetic hydrogel was formed by suspending micron-sized particles of strontium iron oxide in the solution of uncross-linked polymer prior to gelation. The ribbon, made from a mixture of 1% AA and 0.6% ι-carrageenan, was strongly attracted to a bar magnet and could wrap around sharp corners or fold back on itself without fracturing.

Example 3 – Methods of Producing Composite Hydrogels

Composite Hydrogels Containing Different Gelling Agents (Dry Method)

[0088] Shaped hydrogels of Ca^{2+}–AA surrounded by Fe^{3+}–AA were made according to the procedure illustrated in Figure 10. First, a laser cutter was used to cut shaped holes through a piece of chromatography paper with toner printed on one side. The sheet was wet with 1 M $FeCl_3$ solution (on the side that did not contain toner), and a second piece of unpatterned chromatography paper (which did not contain toner or holes) was wet with 2 M $CaCl_2$. The sheets were dried with a heat gun (at about 35 °C), and the sheet with $FeCl_3$ was placed on top of the sheet with $CaCl_2$ such that the toner was sandwiched between the two sheets to prevent aqueous solutions on the sheets from mixing. The desired shape of the perimeter for the film was cut into a sheet of transparency film with the laser cutter, and this sheet was stacked on top of the layered paper template. The stack was held flat against a Petri dish with adhesive (either Scotch® tape or UHU-brand glue stick), and an aqueous solution of sodium alginate (2%, w/w) was poured onto the template. After 2 minutes, the uncross-linked polymer was washed away and a spatula was used to remove the composite film from the template. If fibers of paper adhered to the bottom face of the gel, the film was immersed in water for several minutes until the fibers loosened such that they could be gently rubbed away from the gel by hand. To create the inverse pattern—structures of Fe^{3+}–AA surrounded by Ca^{2+}–AA—the same procedure was carried out except the first (bottom) sheet was wet with 2 M $CaCl_2$ and the second (top) sheet was wet with 1 M $FeCl_3$.

[0089] Figure 11B depicts the resulting hydrogel film made by this method. 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.

[0090] In an analogous procedure, the inverse pattern (structures of Fe^{3+}–AA surrounded by Ca^{2+}–AA, Figure 11A) was made using the same template and switching the solutions used to wet the two layers of the template. The top layer contained 2 M $CaCl_2$ and the bottom layer had 1 M $FeCl_3$. The thickness of the Ca^{2+}–AA regions of the gel was 0.72 ± 0.19 mm, and the thickness of the Fe^{3+}–AA shapes was 0.73 ± 0.08 mm.

Composite Hydrogels Containing Different Gelling Agents (Wet Method)

[0091] A hydrogel patterned into the shape of a star composed of a region of Ca^{2+}–CG and a region of Fe^{3+}–CG was fabricated (see Figure 12). A laser cutter was used to cut a star shape into a sheet of transparency film. Scissors were used to cut a long rectangle of chromatography paper backed with toner on one side. An unpatterned piece of chromatography paper (which did not contain toner or holes) was backed with duct tape and pasted flat against a plastic Petri dish. Paste was applied to the side of the patterned chromatography paper with toner and glued on top of the unpatterned sheet. Finally, the transparency film was pasted on top of the stack. With a 100 μL micropipettor, the bottom layer of paper was wet with aqueous 2 M $FeCl_3$ and the top layer was wet with 2 M $CaCl_2$. An aqueous solution of ι-carrageenan (2%, w/w) poured onto the template gelled into the desired film within 2 minutes, and the excess (uncross-linked) polymer was washed away with tap water. The transparency film was peeled away from the Petri dish and a metal spatula was used to remove the composite film from the template.

[0092] Composite films of ι-carrageenan were also made by the same process. A single hydrogel film was produced using the template shown in Figure 12B. This resulted in a hydrogel of ι-carrageenan in the shape of a star, where the left half was Fe^{3+}–CG and the right half was Ca^{2+}–CG (Figure 12A).

<u>Composite Hydrogels Containing Different Hydrogel Precursors</u>

[0093] Composite hydrogels were made according to the method illustrated in Figure 13. To make shaped hydrogels having Fe^{3+}–AA dyed blue and Fe^{3+}–AA dyed red, surrounded by un-dyed Fe^{3+}–AA, a laser cutter was used to cut circular holes through a piece of chromatography paper with toner printed on one side and a sheet of waxed weighing paper. The chromatography paper was then wetted with 1 M $FeCl_3$ (on the side that did not contain toner) and un-dyed alginic acid was poured onto the template. A gel with shaped holes formed. The uncross-linked polymer was washed away and the gel was kept attached to the paper. A second piece of chromatography paper (which did not contain toner or holes) was wetted with 1 M $FeCl_3$. The gel with was pressed the shaped holes face down against the second sheet of paper, and the waxed weighing paper was pressed against the toner. Reactive Blue 2 (Sigma-Aldrich) and Cibacron Brilliant Red 3B-A (Sigma-Aldrich) dyes were mixed into alginic acid solution at around 1 mg/mL and these colored solutions were poured into the holes. The uncross-linked polymer was washed away and the waxed paper and paper templates were carefully peeled away from the composite gel.

[0094] Figure 14 shows the resulting hydrogel film. The thickness of the undyed Fe^{3+}–AA portion of the gel was 0.42 ± 0.02, the Fe^{3+}–AA dyed blue was 0.45 ± 0.03 mm, and the Fe^{3+}–AA dyed red was 0.48 ± 0.03 mm.

Composite Hydrogels Containing Step-Gradients in Concentration of Gelling Agents:

[0095] Shaped hydrogels of Fe^{3+}–AA were made having varying cross-linking density. A pair of scissors was used to cut a piece of chromatography paper with toner printed on one side into several squares. Each square contained a different concentration of $FeCl_3$ solution: 0.2 M, 0.8 M, or 3.0 M. A template was formed by placing the pieces next to each other in order of increasing ion concentration with a small amount of overlap (about 2 mm). 1.5% AA was poured on top of the template. The uncross-linked polymer was washed away after 2 minutes and a spatula was used to carefully remove the gel from the paper.

[0096] Figure 16 depicts the resulting Fe^{3+}-AA film containing a gradient in concentration of the cross-linking ion, Fe^{3+}. The color of the gel increased from light orange to dark orange as the cross-linking density increased. The thicknesses of the film were 0.18 ± 0.02 mm, 0.61 ± 0.03 mm, and 0.90 ± 0.10 mm for the 0.2 M, 0.8 M, and 3.0 M $FeCl_3$ sections, respectively.

[0097] The mechanical properties of the film varied as a function of the cross-linking density. Figure 16B shows the film placed on a glass pipette. 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 pipette. Conversely, the section of the film cross-linked with the most concentrated solution of Fe^{3+} was the strongest and resisted bending.

pH Sensors

[0098] Shaped hydrogels were fabricated that contained windows of alginate with different pH indicator dyes. A laser cutter was used to cut shaped holes through a piece of chromatography paper (with toner printed on one side) and through a sheet of waxed weighing paper. The chromatography paper was then wetted with 2 M $CaCl_2$ (on the side that did not contain toner) and alginic acid containing 2% activated carbon (Sigma-Aldrich) was poured onto the template.

After 2 minutes, a black gel with shaped holes formed. The uncross-linked polymer was washed away and the gel was kept attached to the paper. A second piece of chromatography paper (this sheet did not contain toner or holes) was wetted with 2 M $CaCl_2$. The gel was pressed with the shaped holes face down onto the second sheet of paper and the waxed weighing paper was pressed against the toner. Thymol blue indicator (Harleco) and bromocresol green indicator (LaMotte Chemical) were dissolved in methanol and mixed into the alginic acid solution. These colored solutions were poured into the holes. After 2 minutes, the uncross-linked polymer was washed away and the waxed paper and paper templates were carefully peeled away from the composite gel.

[0099] The composite gels changed colors with changes in pH. In strong acid (3 M HCl), the thymol blue compartment turned red while the bromocresol green compartment turned yellow. At pH 7, the thymol blue compartment turned yellow while the bromocresol green compartment turned blue.

Example 4 – Analysis of Bacterial Growth in Shaped Hydrogels

Reagents

[0100] Stock solutions were made from deionized water unless otherwise indicated. Liquid LB medium: 5 g/L yeast extract, 10 g/L peptone from casein, 5 g/L NaCl. Isopropyl β-D-1-thiogalactopyranoside (IPTG): 48 mg/mL (0.2 M). X-gal (5-bromo-4-chloro-3-indolyl-β-D-galactopyranoside): 80 mg/mL (0.2 M) in *N*,*N*-Dimethylformamide (DMF). LB-agar plates were poured from an autoclaved solution of 20 g/L agar in LB medium to which 100 mg/L of sodium ampicillin was added after the solution had cooled below 55 °C.

[0101] *Escherichia coli* BL21gold(DE3) (Stratagene) were transformed with the plasmid pUC18 (Stratagene, La Jolla, California) using the protocol supplied by the vendor. 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. The bacteria were cultured in liquid LB and on LB-agar plates at 37 °C.

[0102] The growth of bacteria was tested in homogeneous films of Ca^{2+}–AA, Ba^{2+}–AA, Ni^{2+}–AA, Cu^{2+}–AA, Al^{3+}–AA, Ho^{3+}–AA, and Gd^{3+}–AA. The polymer stock solution used to make the films contained AA (20 g/L), yeast extract (10 g/L), peptone from casein (20 g/L), and NaCl (10 g/L). Prior to formation of the films, 500 µL of 0.2 M IPTG and 500 µL of 0.2 M X-gal were added to a 35 mL aliquot of the solution of AA–LB and the mixture was shaken vigorously. Once the air bubbles in the solution had dissipated, 3 mL of a dense liquid culture of *E. coli* was added and the mixture was rocked gently so as not to introduce new bubbles of air. Circular films were made from this solution, placed in polystyrene culture trays, incubated for 6 hr at 37 °C, and photographed.

[0103] For screening the toxicity of homogeneous films of M^{n+}–AA, the films were formed using solutions that were 1 M in $CaCl_2$ and 1 M in $Ni(NO_3)_2$, $Cu(NO_3)_2$, $BaCl_2$, $Al(NO_3)_3$, $Ho(NO_3)_3$, and $GdCl_3$. As control experiments, films were also formed with 2 M $CaCl_2$ and 2 M $Ca(NO_3)_3$ to demonstrate that the toxicity of the cross-linking solutions arose from the metal ions and not the counteranions.

[0104] The growth of bacteria was tested on composite gels of AA cross-linked with aluminum ions and calcium ions. To create a square structure of Al^{3+}–AA + Ca^{2+}–AA surrounded by a hexagon of Ca^{2+}–AA, a laser cutter was used to cut a square hole through a piece of chromatography paper with toner printed on one side and to cut a hexagon-shaped hole into a sheet of transparency film. In sequence, a sheet of unpatterned chromatography paper was glued to a Petri dish, the patterned piece of chromatography paper was glued on top of the first sheet, and the transparency film was glued on top of the stack, resulting in the template depicted in Figure 17A. The bottom piece of paper was wet with a solution of 1 M $Al(NO_3)_3$ + 1 M $CaCl_2$, and the top sheet was wet with 2 M $CaCl_2$. The AA–LB solution was poured onto this new template, and 2 minutes later, the uncross-linked polymer was washed away. A spatula was used to peel the composite gel from the template.

Controlled Growth of Bacteria

[0105] The viability of *E. coli* in homogeneous alginate films cross-linked by different metals was tested. 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 (Harrison et al., Bmc Microbiology 5:11 (2005)). 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, solutions of 1 M $CaCl_2$ and 1 M of a toxic metal were used. These films could be manipulated without tearing, yet they were still toxic to *E. coli*.

[0106] Figure 17 depicts the culture of *E. coli* in a composite hexagon-shaped film of Ca^{2+}–AA with a central square-shaped region of Al^{3+}–AA + Ca^{2+}–AA. After incubation for 6 hr at 37 °C, the bacteria grew almost exclusively in the region of gel cross-linked by calcium ions, as indicated by the formation of blue pigment in the outer region of the hexagon. The aluminum ions in the central region of the film inhibited growth of bacteria such the square region remained uncolored.

[0107] 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 travelling through a stationary phase of AA, followed the order: $Pb^{2+} > Ba^{2+} > Fe^{3+}, Al^{3+} > Cu^{2+}, Cd^{2+}, Ho^{3+} > Ca^{2+} > Zn^{2+}, Co^{2+}, Ni^{2+} > Mn^{2+}, Mg^{2+}$ (Cozzi et al., J. Chromatogr. 35:396-404 (1968); Cozzi et al., J. Chromatogr. 35:405-415 (1968); Muzzarelli, Natural Chelating Polymers; Pergamon Press: Oxford (1973); Winkleman et al., Chem. Mater.19:1362-1368 (2007)). For metals with low affinities, the ions can spread away from their original regions of gel and kill bacteria in regions intended to have viable colonies. Metals with high affinities for AA (e.g., Al^{3+}) tended not to spread around the film such that the desired pattern was produced with greater fidelity.

EQUIVALENTS

[0108] It is to be understood that while the invention has been described in conjunction with the detailed description thereof, the foregoing description is intended to illustrate and not limit the scope of the invention, which is defined by the scope of the appended claims. Other aspects, advantages, and modifications are within the scope of the following claims.

CLAIMS

1. A method of making a shaped hydrogel, comprising:

providing a template comprising a porous, hydrophilic region bounded by a fluid-impermeable barrier, the barrier substantially permeating the thickness of the porous, hydrophilic template;

contacting the porous, hydrophilic region of the template with an aqueous solution comprising a gelling agent, wherein the porous, hydrophilic region absorbs the solution; and

contacting the porous, hydrophilic region of the template with a hydrogel precursor, wherein the gelling agent crosslinks the hydrogel precursor and forms a hydrogel.

2. The method of claim 1, wherein the hydrogel has the shape of the porous, hydrophilic region.

3. The method of claim 1, wherein the template comprises paper.

4. The method of claim 1, wherein the template comprises chromatography paper.

5. The method of claim 1, wherein the gelling agent is a multivalent cation.

6. The method of claim 1, wherein the cross-linking reagent is a multivalent anion.

7. The method of claim 1, wherein the hydrogel precursor is alginic acid (AA), carboxymethylcellulose (CMC), ι-carrageenan, poly(galacturonic acid) (PG), or poly(bis(4-carboxyphenoxy)-phosphazene.

8. The method of claim 5, wherein the cation is Pb^{2+}, Ba^{2+}, Fe^{3+}, Al^{3+}, Cu^{2+}, Cd^{2+}, Ho^{3+}, Ca^{2+}, Zn^{2+}, Co^{2+}, Ni^{2+}, Mn^{2+}, or Mg^{2+}, Sr^{2+}, Gd^{3+}, Pb^{2+}, Ra^{2+}, Fe^{2+}, Pd^{2+}, Bi^{3+}, Hg^{2+}, Au^{3+}, Co^{2+}, Co^{3+}, Cr^{2+}, Cr^{3+}, Mn^{4+}, Pt^{2+}, Pt^{4+}, Sn^{2+}, Sn^{4+}, Ce^{3+}, Ce^{4+}, Ga^{3+}, V^{3+}, or Rh^{3+}.

9. The method of claim 1, wherein the barrier comprises toner ink.

10. The method of claim 1, wherein the barrier comprises photoresist.

11. The method of claim 1, wherein the barrier comprises a plastic sheet or film disposed on the surface of the template.

12. The method of claim 11, wherein the plastic sheet comprises adhesive tape.

13. A method of making a shaped composite hydrogel, comprising:

providing a first template comprising a porous, hydrophilic sheet and a fluid-impermeable layer disposed the hydrophilic sheet; wherein said first template comprises a shape cut out of the template;

providing a second template comprising a porous, hydrophilic sheet;

contacting the porous, hydrophilic sheet of the first template with a first aqueous solution comprising a first gelling agent;

contacting the porous, hydrophilic sheet of the second template with a second aqueous solution comprising a second gelling agent;

contacting the first template on top of the second template such that the fluid-impermeable layer of the first template contacts the porous, hydrophilic sheet of the second template and at least a portion of the porous, hydrophilic sheet of the second template is exposed through the shape cut of the first template; and

contacting the porous, hydrophilic sheet of the first template and the exposed portion of the porous, hydrophilic sheet of the second template with a hydrogel precursor, wherein the first gelling agent crosslinks the hydrogel precursor and forms a first type of hydrogel and the second gelling agent crosslinks the hydrogel precursor and forms a second type of hydrogel.

14. The method of claim 13, wherein the second type of hydrogel has the same shape as the shape cut of the first template.

15. The method of claim 13, wherein the first type of hydrogel has the same shape as the inverse of the shape cut out of the first template.

16. The method of claim 13, wherein the first type of hydrogel and the second type of hydrogel form a continuous composite hydrogel.

17. The method of claim 13, wherein the first template and the second template comprise paper.

18. The method of claim 13, wherein the first template and the second template comprise chromatography paper.

19. The method of claim 13, wherein the hydrogel precursor is alginic acid (AA), carboxymethylcellulose (CMC), ι-carrageenan, poly(galacturonic acid) (PG), or poly(bis(4-carboxyphenoxy)-phosphazene.

20. The method of claim 19, wherein the hydrogel precursor comprises alginic acid.

21. The method of claim 20, wherein the first gelling agent is Ca^{2+} and the second gelling agent is Fe^{3+}.

22. The method of claim 13, wherein the fluid-impermeable layer of the first template comprises toner ink.

23. The method of claim 13, wherein the fluid-impermeable layer comprises photoresist.

24. The method of claim 13, wherein the fluid-impermeable layer comprises a plastic sheet.

25. The method of claim 24, wherein the plastic sheet comprises adhesive tape.

26. A method of making a shaped composite hydrogel, comprising:

providing a first template comprising a porous, hydrophilic sheet and a fluid-impermeable layer disposed on the hydrophilic sheet; wherein said first template comprises a shape cut out of the template;

providing a second template comprising a porous, hydrophilic sheet;

contacting the porous, hydrophilic sheet of the first template and the porous, hydrophilic sheet of the second template with an aqueous solution comprising a gelling agent;

contacting the porous, hydrophilic sheet of the first template with a first hydrogel precursor, wherein the gelling agent crosslinks the first hydrogel precursor and forms a first hydrogel comprising a shape that is the same as the shape cut out of the template;

contacting the first template comprising the first hydrogel with the second template such that the first hydrogel contacts the porous, hydrophilic sheet of the second template and at least a portion of the porous, hydrophilic sheet of the second template is exposed through the shape cut out of the fluid-impermeable layer of the first template; and contacting the exposed portion of the porous, hydrophilic sheet of the second template with a second hydrogel precursor, wherein the gelling agent from the porous, hydrophilic sheet of the second template crosslinks the second hydrogel precursor and forms a second hydrogel.

27. The method of claim 26, wherein the second hydrogel has the same shape as the shape cut out of the first template.

28. The method of claim 26, wherein the first hydrogel has the same shape as the inverse of the shape cut out of the first template.

29. The method of claim 26, wherein the first hydrogel and the second hydrogel form a continuous composite hydrogel.

30. The method of claim 26, wherein the first template and the second template comprise paper.

31. The method of claim 26, wherein the first template and the second template comprise chromatography paper.

32. The method of claim 26, wherein the gelling agent is Pb^{2+}, Ba^{2+}, Fe^{3+}, Al^{3+}, Cu^{2+}, Cd^{2+}, Ho^{3+}, Ca^{2+}, Zn^{2+}, Co^{2+}, Ni^{2+}, Mn^{2+}, or Mg^{2+}, Sr^{2+}, Gd^{3+}, Pb^{2+}, Ra^{2+}, Fe^{2+}, Pd^{2+}, Bi^{3+}, Hg^{2+}, Au^{3+}, Co^{2+}, Co^{3+}, Cr^{2+}, Cr^{3+}, Mn^{4+}, Pt^{2+}, Pt^{4+}, Sn^{2+}, Sn^{4+}, Ce^{3+}, Ce^{4+}, Ga^{3+}, V^{3+}, or Rh^{3+}.

33. The method of claim 32, wherein the gelling agent is Fe^{3+}.

34. The method of claim 26, wherein the fluid-impermeable layer of the first template comprises toner ink.

35. The method of claim 26, wherein the fluid-impermeable layer comprises photoresist.

36. The method of claim 26, wherein the fluid-impermeable layer comprises a plastic sheet.

37. The method of claim 36, wherein the plastic sheet comprises adhesive tape.

38. A method of making a composite hydrogel, comprising:

providing a first template comprising a porous, hydrophilic sheet and a fluid-impermeable layer disposed on the hydrophilic sheet;

providing a second template comprising a porous, hydrophilic sheet and a fluid-impermeable layer disposed on the hydrophilic sheet;

contacting the porous, hydrophilic sheet of the first template with a first solution comprising a first concentration of a gelling agent;

contacting the porous, hydrophilic sheet of the second template with a second solution comprising a second concentration of thegelling agent;

joining the first template and the second template end to end such that the porous, hydrophilic sheet of the first template and the porous, hydrophilic sheet of the second template form a continuous top face; and

contacting the top face with a hydrogel precursor, wherein the gelling agent from the first template forms a first hydrogel having a first concentration of gelling agent, and the gelling agent from the second template forms a second hydrogel having a second concentration of gelling agent.

39. The method of claim 38, wherein the first hydrogel and the second hydrogel form a continuous composite hydrogel.

40. The method of claim 38, wherein the first template and the second template comprise paper.

41. The method of claim 38, wherein the first template and the second template comprise chromatography paper.

42. The method of claim 38, wherein the hydrogel precursor is alginic acid (AA), carboxymethylcellulose (CMC), ι-carrageenan, poly(galacturonic acid) (PG), or poly(bis(4-carboxyphenoxy)-phosphazene.

43. The method of claim 3638 wherein the gelling agent is Pb^{2+}, Ba^{2+}, Fe^{3+}, Al^{3+}, Cu^{2+}, Cd^{2+}, Ho^{3+}, Ca^{2+}, Zn^{2+}, Co^{2+}, Ni^{2+}, Mn^{2+}, or Mg^{2+}, Sr^{2+}, Gd^{3+}, Pb^{2+},

Ra^{2+}, Fe^{2+}, Pd^{2+}, Bi^{3+}, Hg^{2+}, Au^{3+}, Co^{2+}, Co^{3+}, Cr^{2+}, Cr^{3+}, Mn^{4+}, Pt^{2+}, Pt^{4+}, Sn^{2+}, Sn^{4+}, Ce^{3+}, Ce^{4+}, Ga^{3+}, V^{3+}, or Rh^{3+}.

44. The method of claim 38, wherein the fluid-impermeable layer of the first template and the fluid-impermeable layer of the second template comprise toner ink.

45. The method of claim 38, wherein the fluid-impermeable layer of the first template and the fluid-impermeable layer of the second template comprise photoresist.

46. The method of claim 38, wherein the fluid-impermeable layer of the first template and the fluid-impermeable layer of the second template comprise a plastic sheet.

47. The method of claim 4446 wherein the plastic sheet comprises adhesive tape.

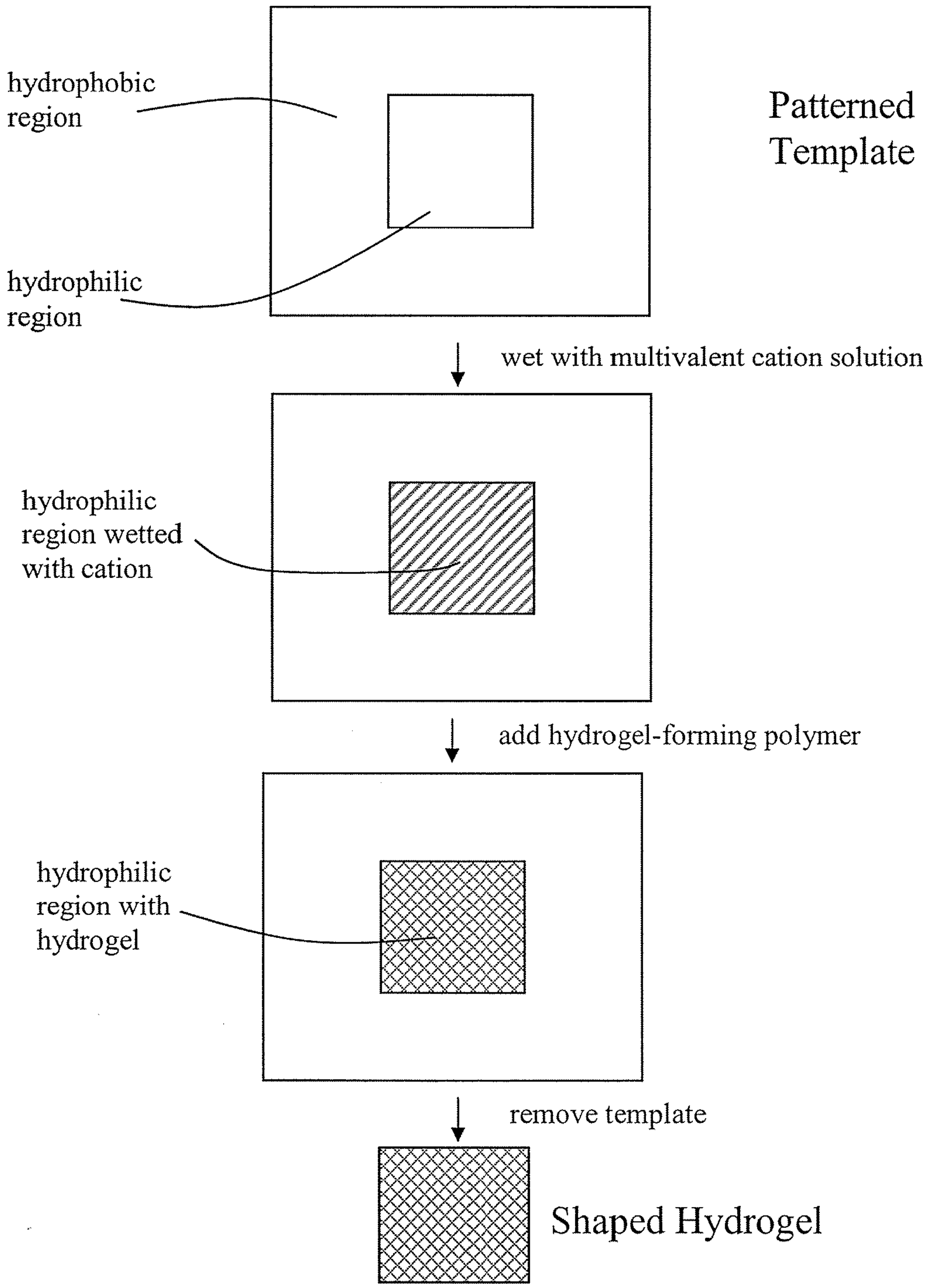

hydrophobic region
Patterned Template
hydrophilic region
wet with multivalent cation solution
hydrophilic region wetted with cation
add hydrogel-forming polymer
hydrophilic region with hydrogel
remove template
Shaped Hydrogel

FIG. 1

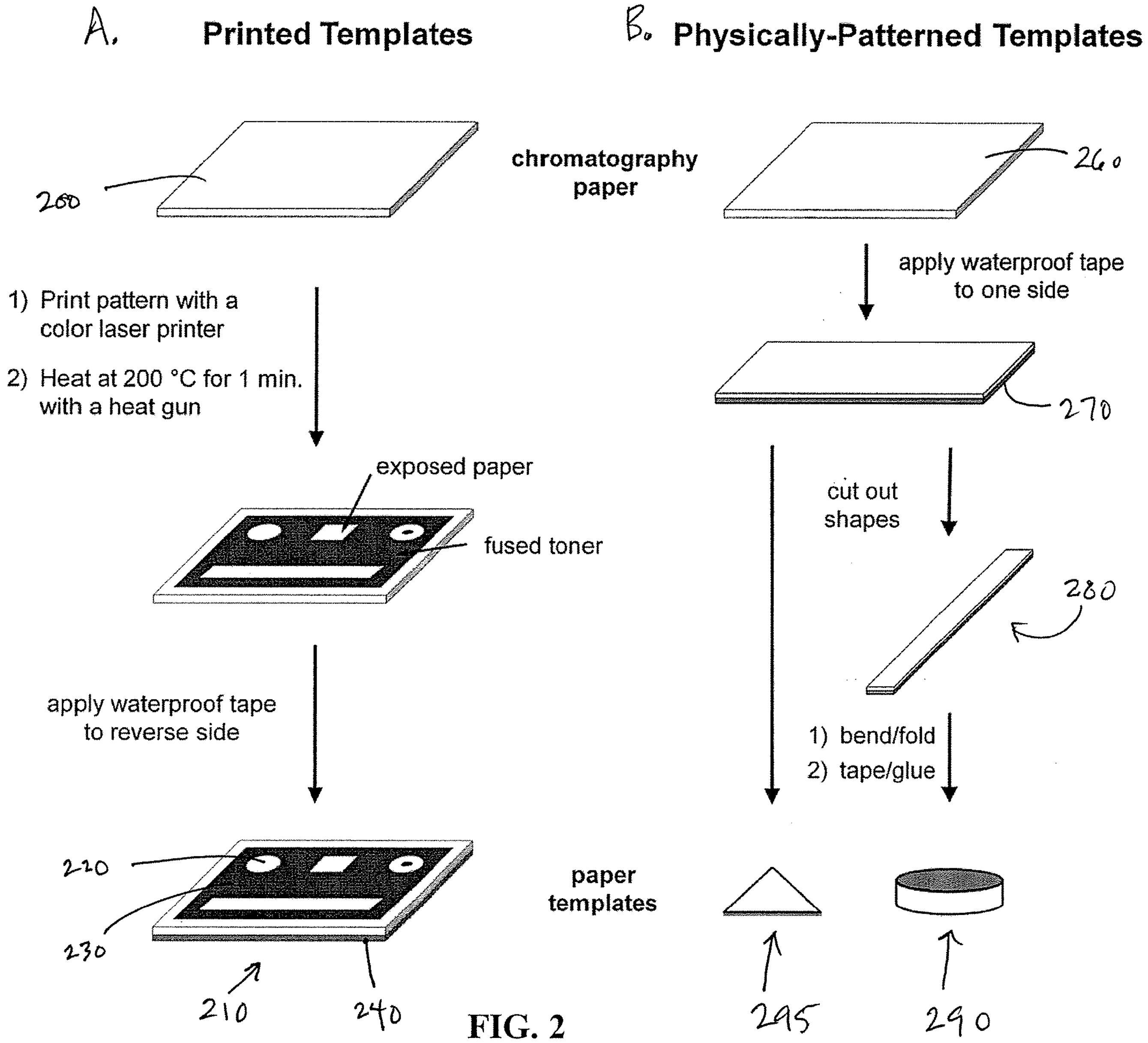

A.
Printed Templates
B.
Physically-Patterned Templates
chromatography paper
200
260
1) Print pattern with a color laser printer
2) Heat at 200 °C for 1 min. with a heat gun
apply waterproof tape to one side
270
exposed paper
fused toner
cut out shapes
280
apply waterproof tape to reverse side
1) bend/fold
2) tape/glue
220
230
210
240
paper templates
295
290

FIG. 2

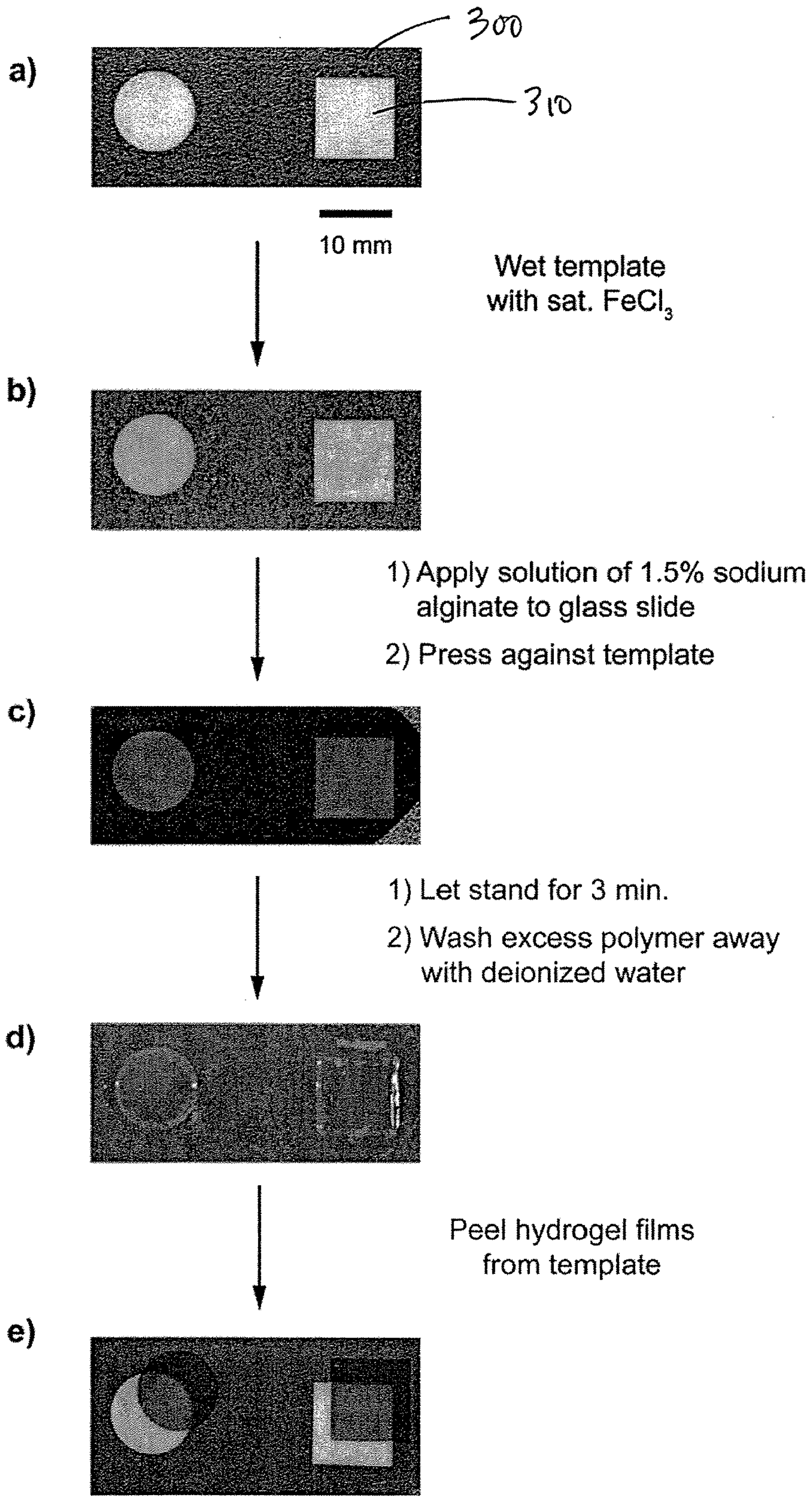

a)
300
310
10 mm
Wet template
with sat. $FeCl_3$
b)
1) Apply solution of 1.5% sodium
alginate to glass slide
2) Press against template
c)
1) Let stand for 3 min.
2) Wash excess polymer away
with deionized water
d)
Peel hydrogel films
from template
e)

FIG. 3

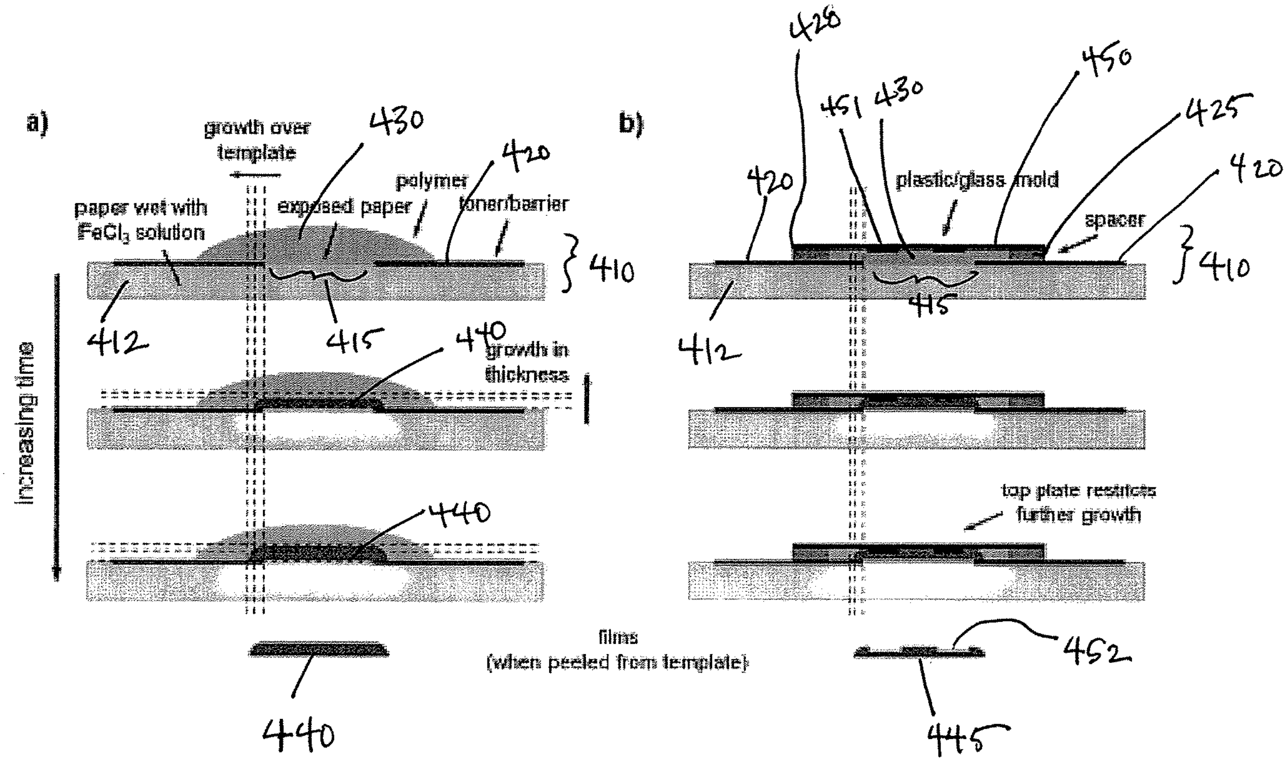

a)
growth over template
430
420
polymer
paper wet with FeCl$_3$ solution
exposed paper
toner/barrier
410
412
415
440
growth in thickness
increasing time
440
films (when peeled from template)
440
b)
428
451
430
450
425
420
plastic/glass mold
420
spacer
410
412
415
top plate restricts further growth
452
445

FIG. 4

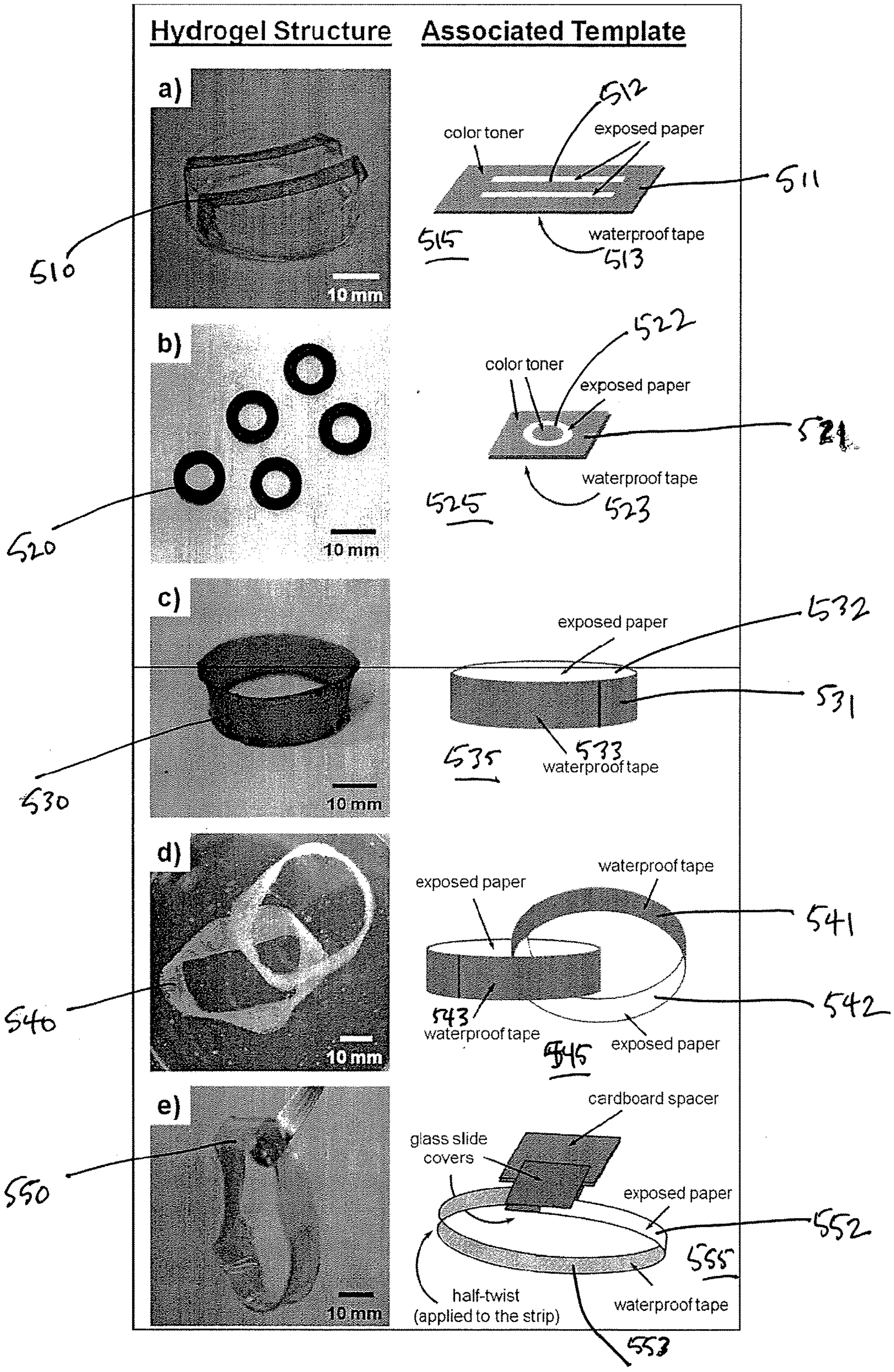

Hydrogel Structure
Associated Template
a)
510
10 mm
512
color toner
exposed paper
511
515
waterproof tape
513
b)
520
10 mm
522
color toner
exposed paper
521
525
waterproof tape
523
c)
530
10 mm
532
exposed paper
531
535
533
waterproof tape
d)
540
10 mm
exposed paper
waterproof tape
541
542
543
waterproof tape
545
exposed paper
e)
550
10 mm
cardboard spacer
glass slide covers
exposed paper
552
555
half-twist (applied to the strip)
waterproof tape
553

FIG. 5

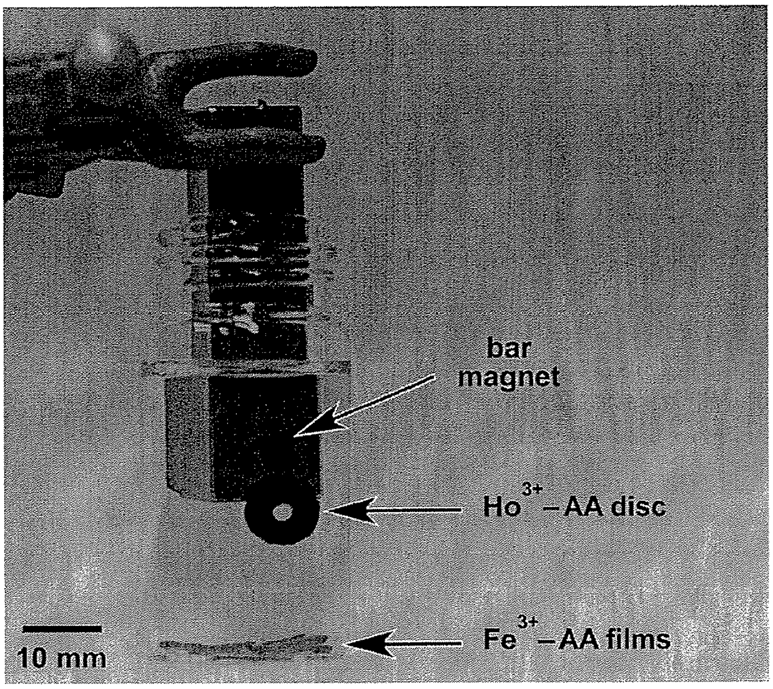


FIG. 6

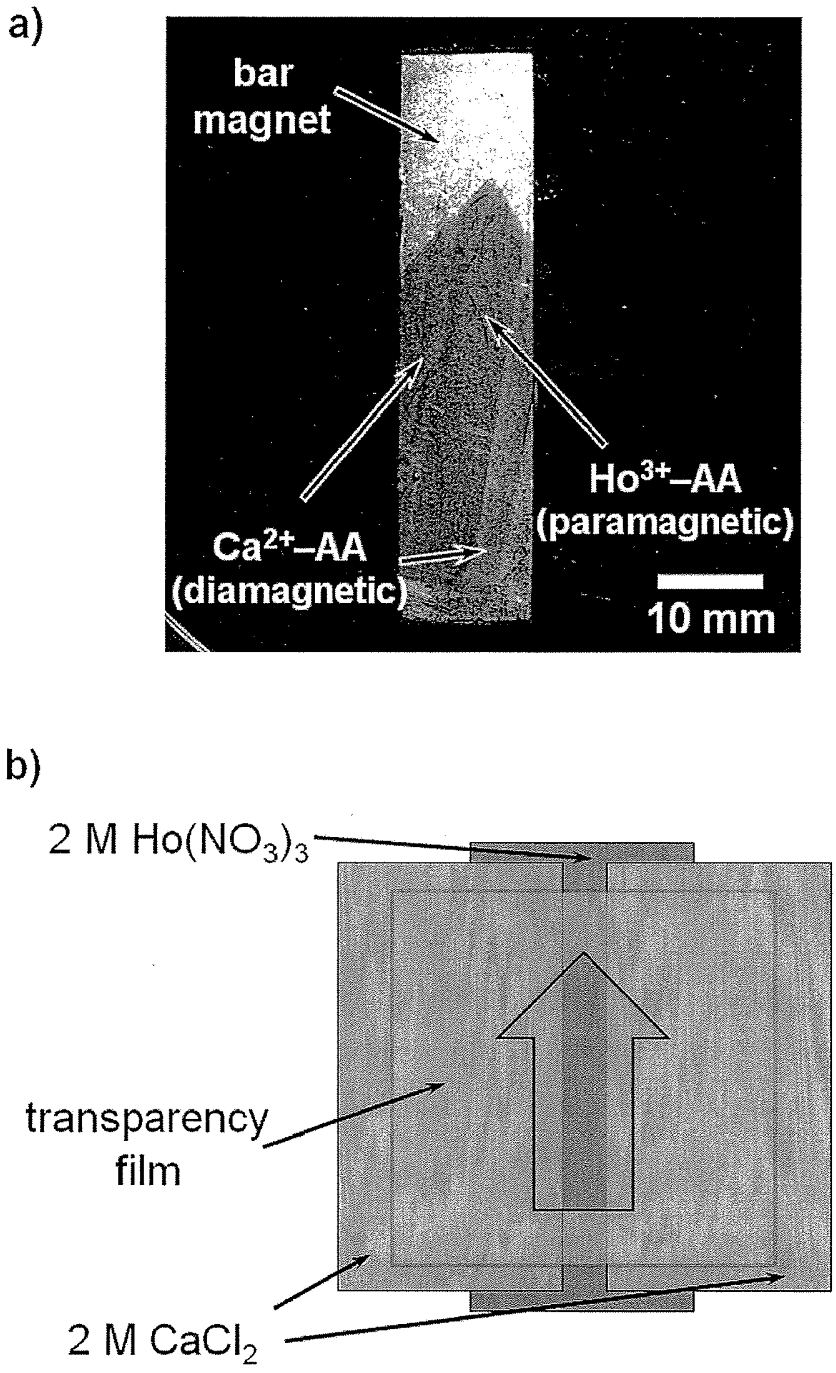
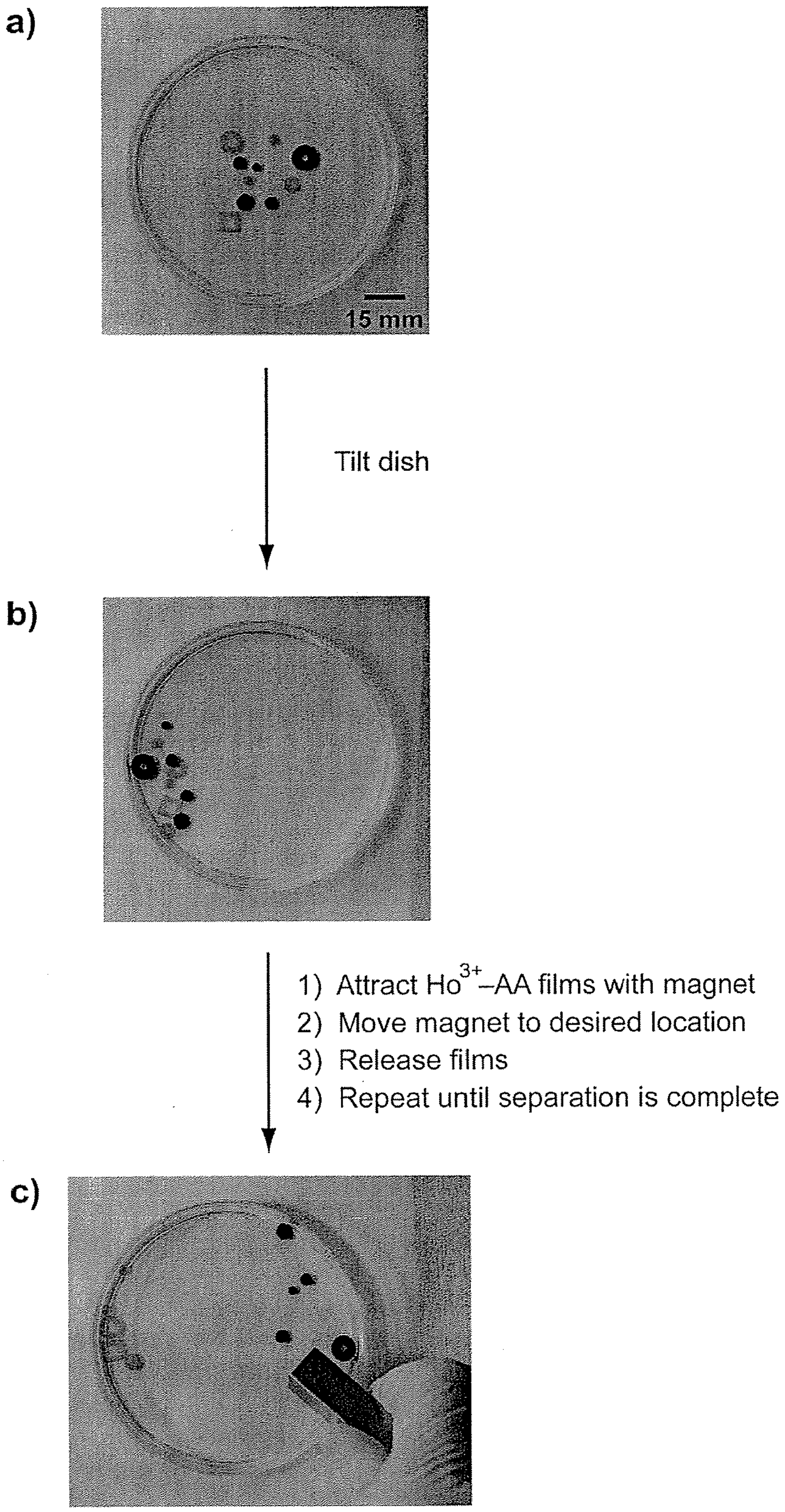

a)
bar magnet
Ho^{3+}–AA (paramagnetic)
Ca^{2+}–AA (diamagnetic)
10 mm
b)
2 M Ho(NO$_3$)$_3$
transparency film
2 M CaCl$_2$

FIG. 7



Tilt dish

1) Attract Ho^{3+}–AA films with magnet
2) Move magnet to desired location
3) Release films
4) Repeat until separation is complete

FIG. 8

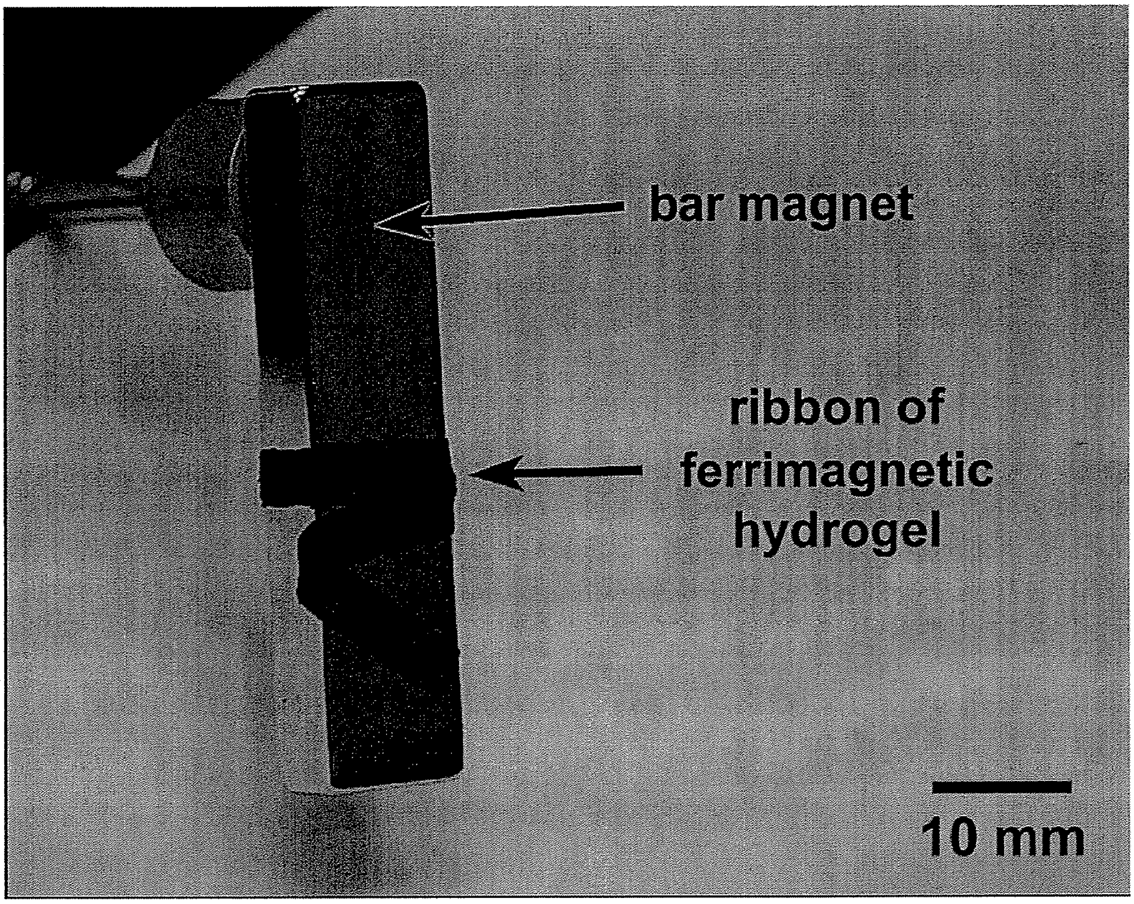


FIG. 9

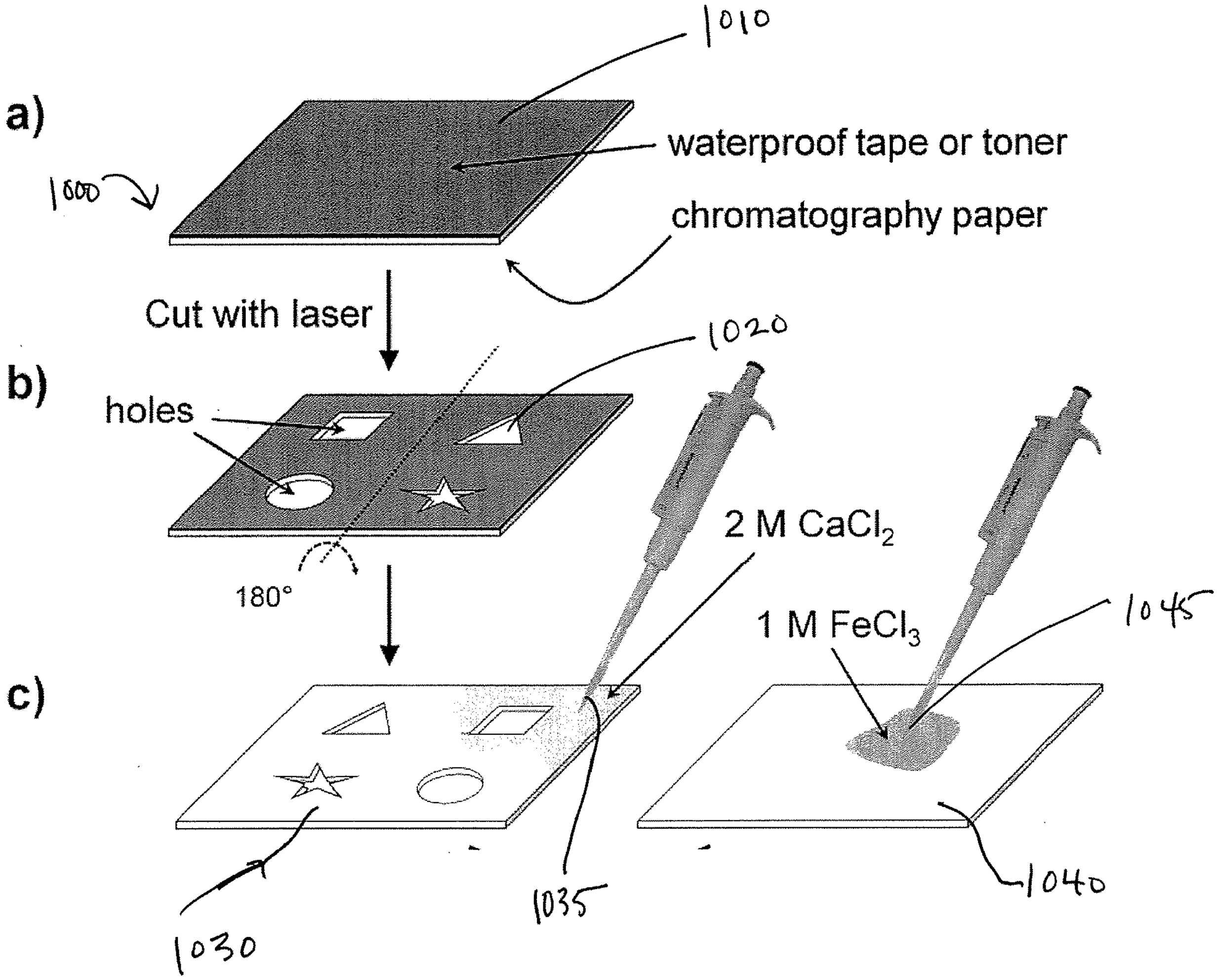

1010
a)
1000
waterproof tape or toner
chromatography paper
Cut with laser
1020
b)
holes
180°
2 M $CaCl_2$
1 M $FeCl_3$
1045
c)
1035
1040
1030

FIG. 10

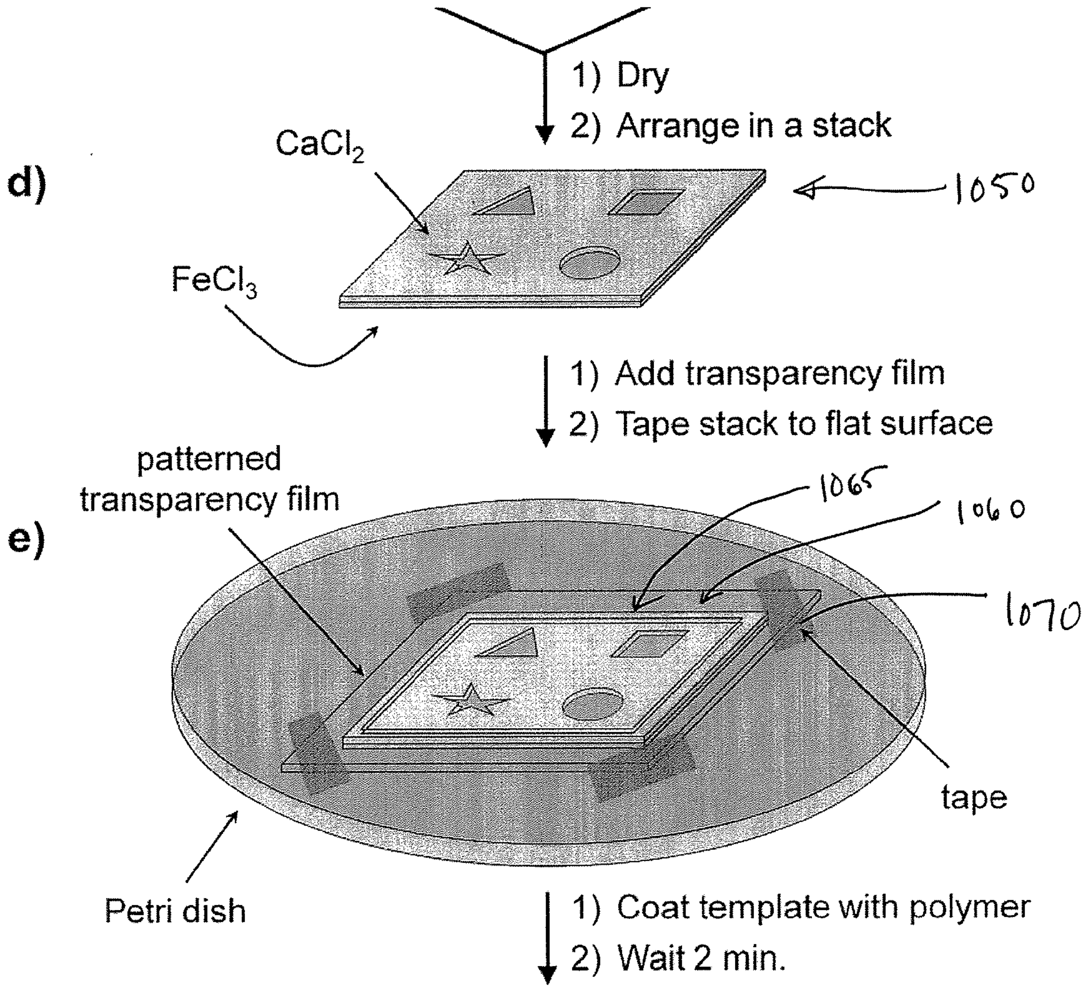

1) Dry
2) Arrange in a stack
d)
CaCl2
1050
FeCl3
1) Add transparency film
2) Tape stack to flat surface
e)
patterned transparency film
1065
1060
1070
tape
Petri dish
1) Coat template with polymer
2) Wait 2 min.

FIG. 10

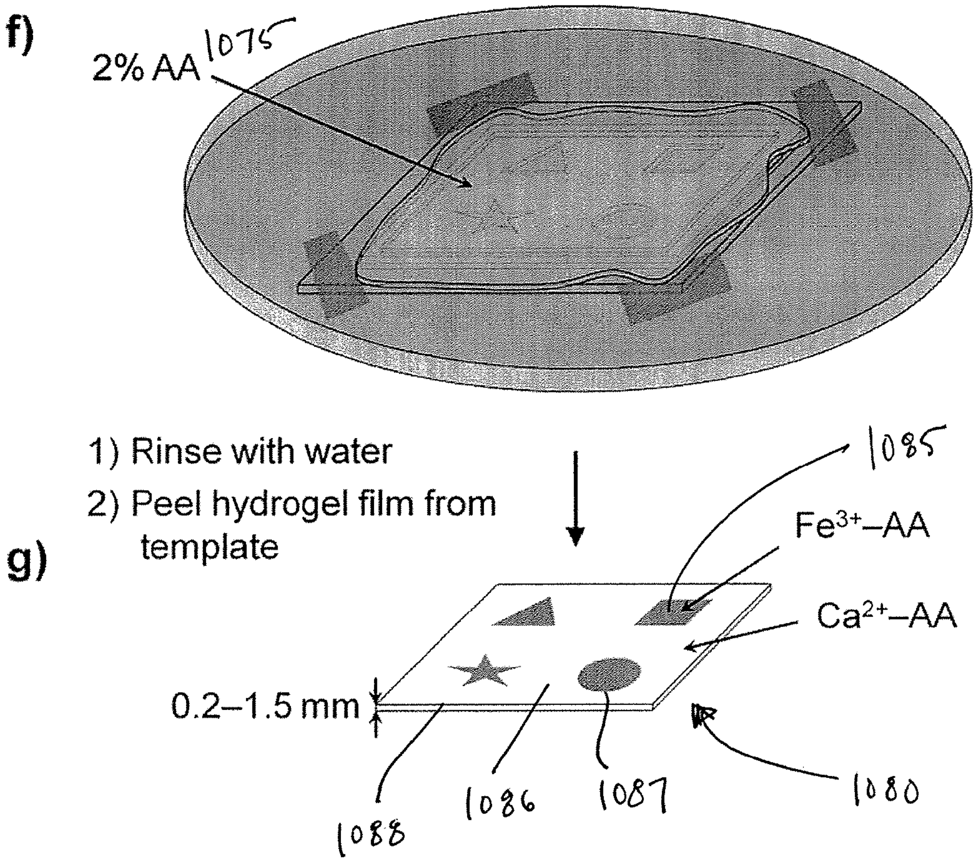

f)
2% AA
1075
1) Rinse with water
2) Peel hydrogel film from template
g)
1085
Fe3+–AA
Ca2+–AA
0.2–1.5 mm
1088
1086
1087
1080

FIG. 10

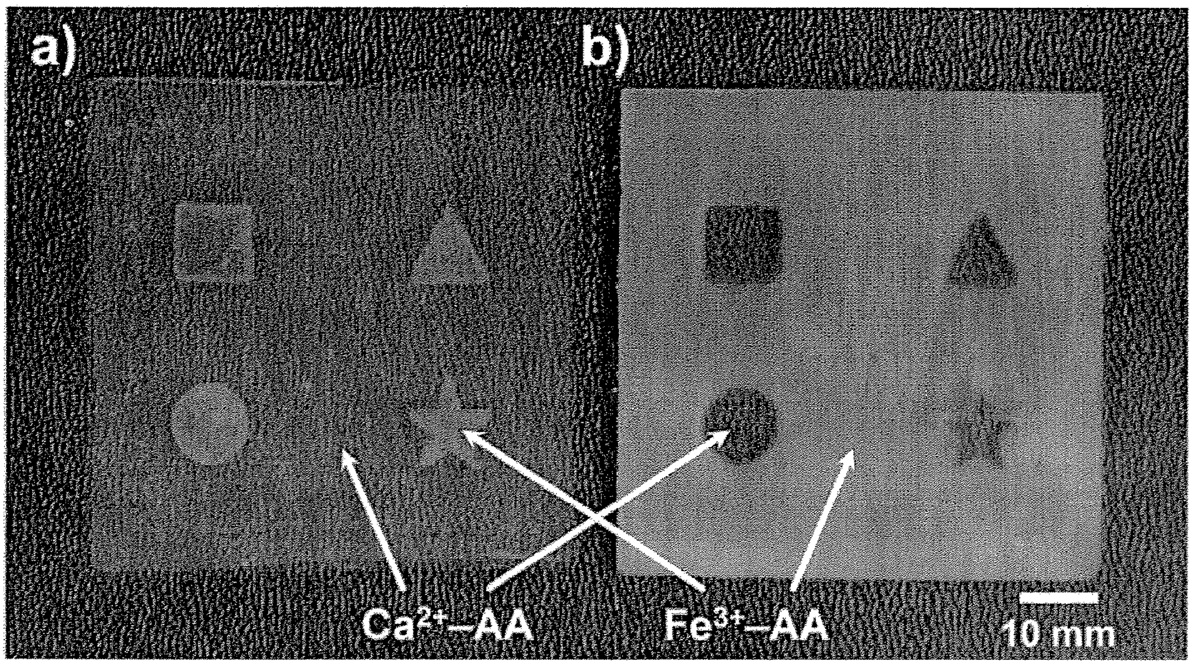


FIG. 11

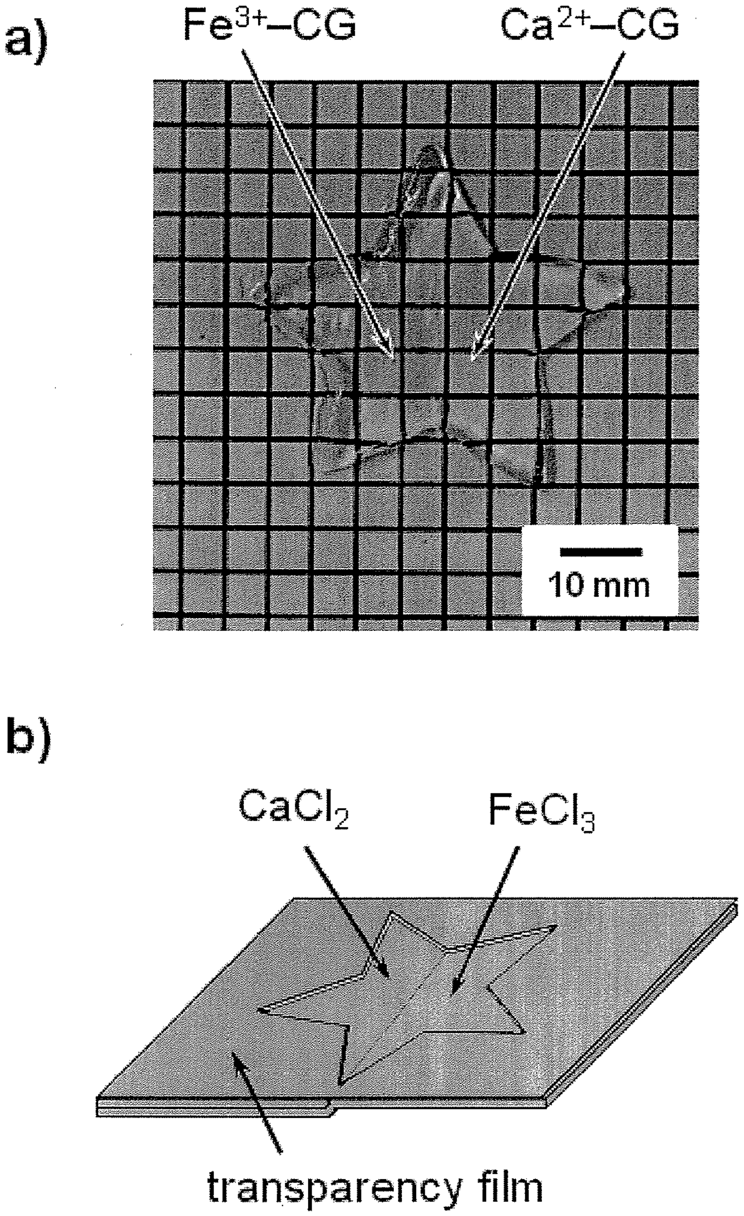


FIG. 12

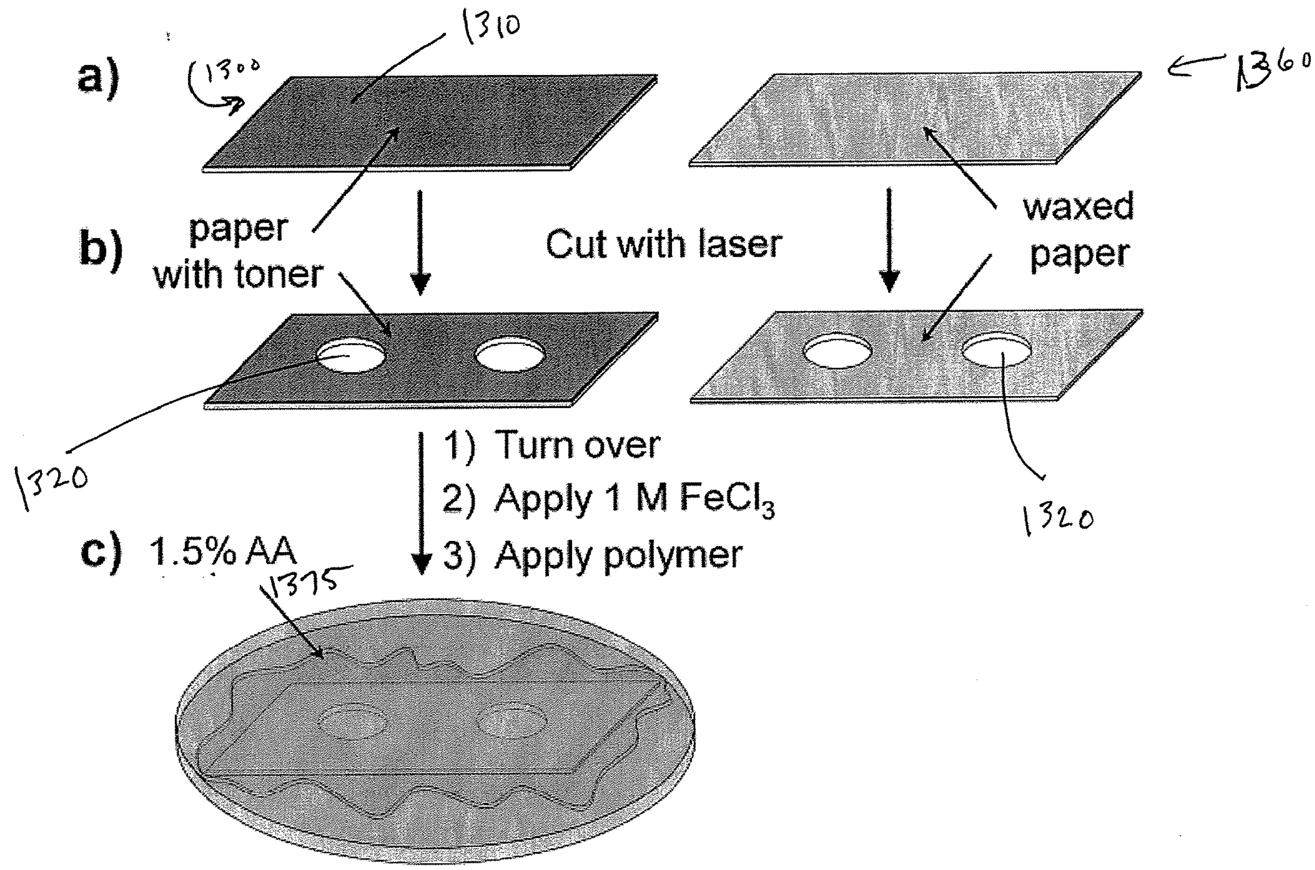

a)
1300
1310
1360
paper with toner
b)
Cut with laser
waxed paper
1320
1320
1) Turn over
2) Apply 1 M $FeCl_3$
3) Apply polymer
c) 1.5% AA
1375

FIG. 13

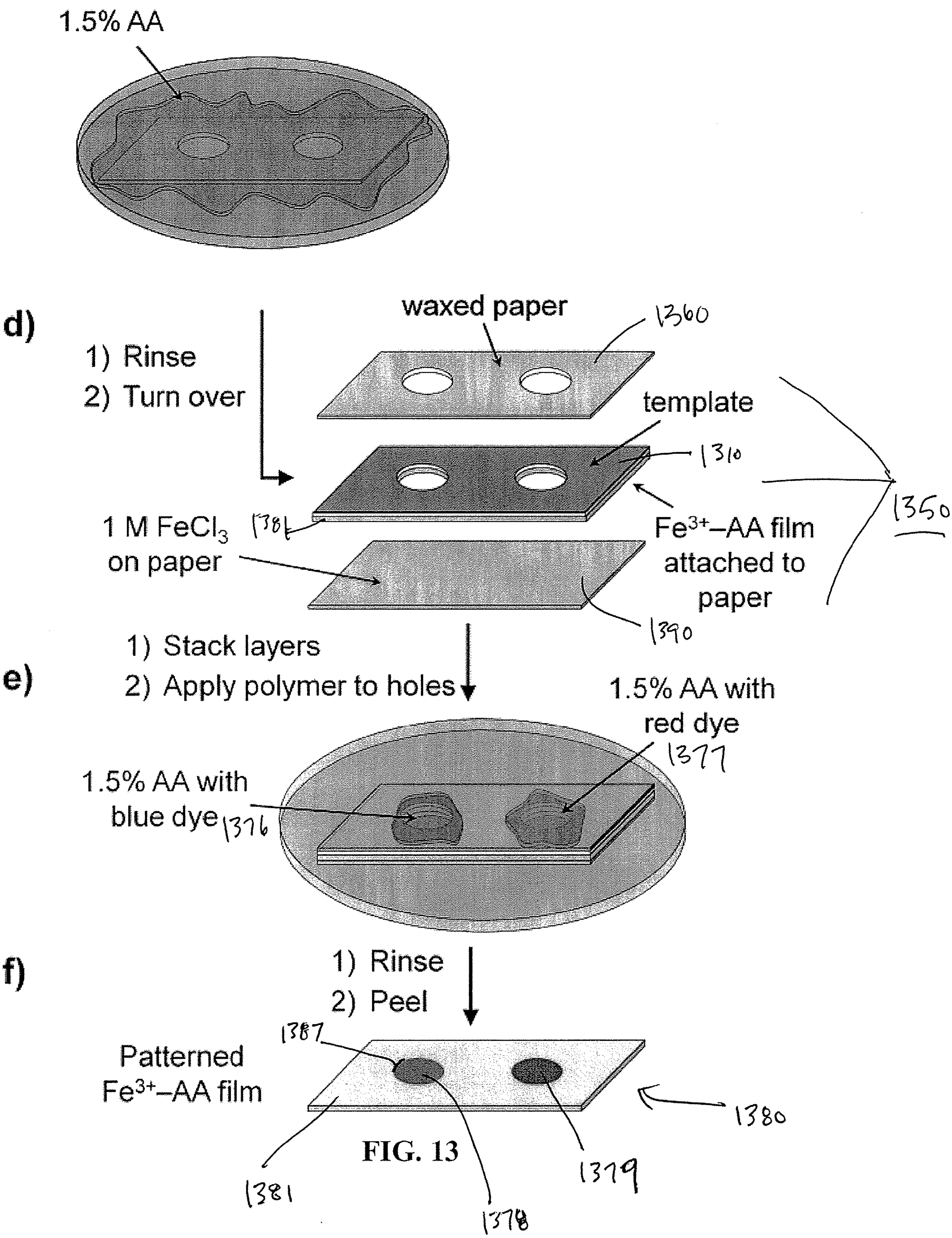

1.5% AA
d)
1) Rinse
2) Turn over
waxed paper
1360
template
1310
1350
1 M FeCl$_3$
on paper
1386
Fe^{3+}–AA film
attached to
paper
1390
1) Stack layers
e)
2) Apply polymer to holes
1.5% AA with
red dye
1377
1.5% AA with
blue dye
1376
f)
1) Rinse
2) Peel
1387
Patterned
Fe^{3+}–AA film
1380
1381
1379
1378

FIG. 13

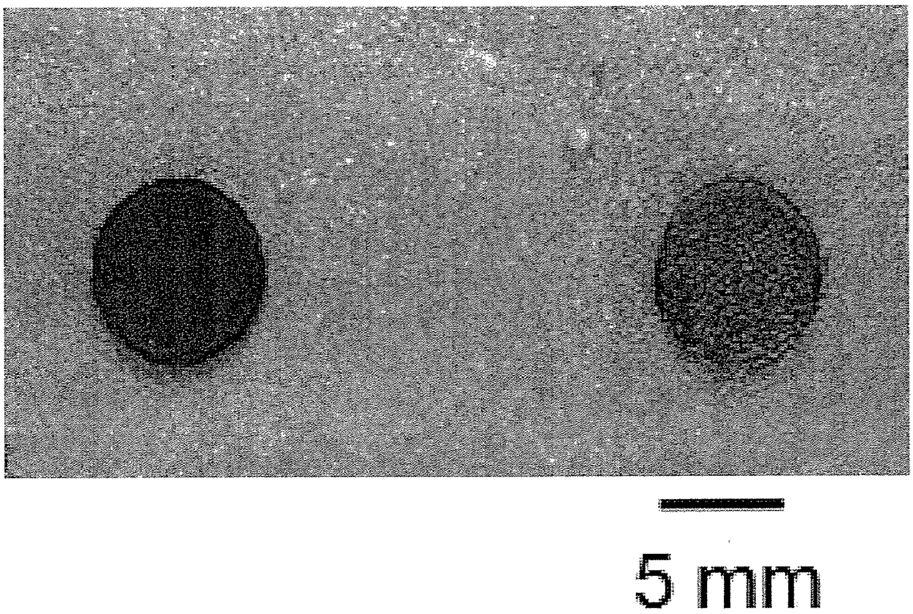
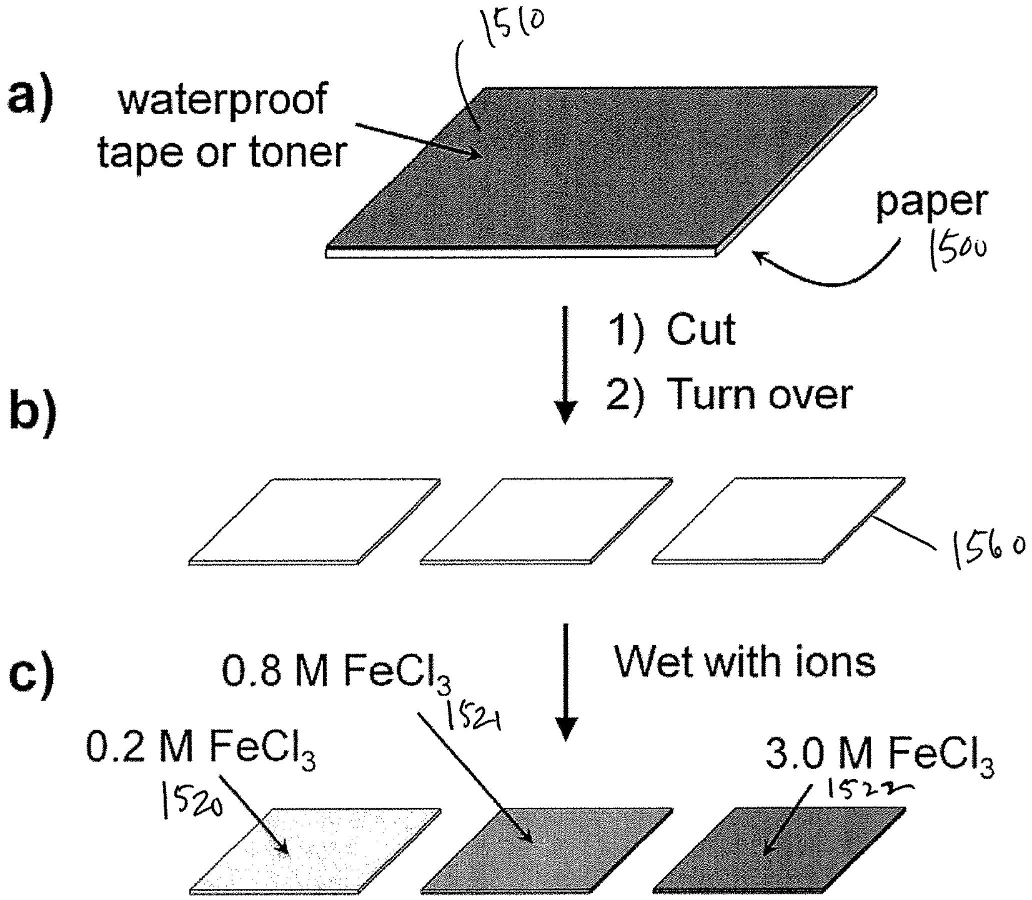


FIG. 14



1510
a)
waterproof tape or toner
paper
1500
1) Cut
2) Turn over
b)
1560
c)
Wet with ions
0.8 M FeCl$_3$
1521
0.2 M FeCl$_3$
1520
3.0 M FeCl$_3$
1522

FIG. 15

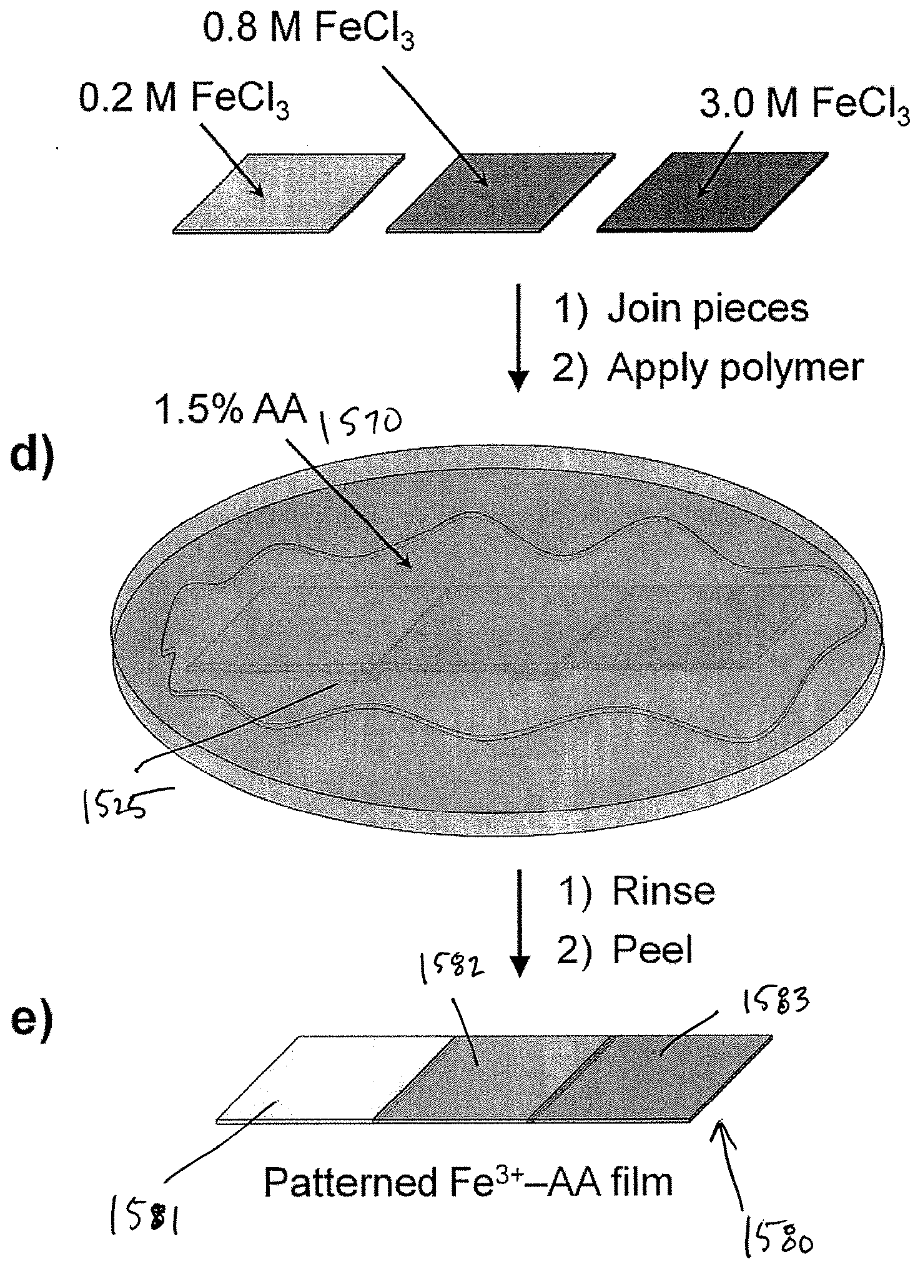

0.8 M $FeCl_3$
0.2 M $FeCl_3$
3.0 M $FeCl_3$
1) Join pieces
2) Apply polymer
d)
1.5% AA
1510
1525
1) Rinse
2) Peel
e)
1582
1583
1581
Patterned Fe^{3+}–AA film
1580

FIG. 15

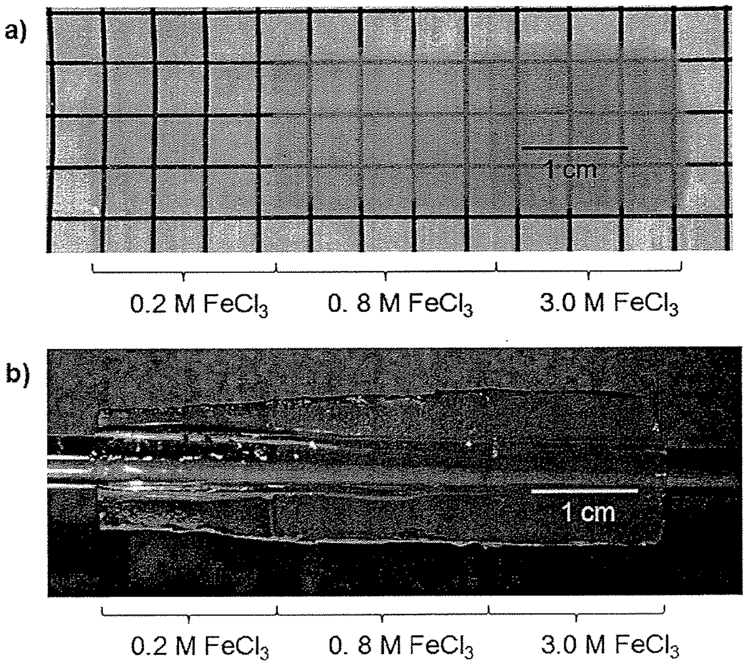

a)
1 cm
0.2 M $FeCl_3$
0. 8 M $FeCl_3$
3.0 M $FeCl_3$
b)
1 cm
0.2 M $FeCl_3$
0. 8 M $FeCl_3$
3.0 M $FeCl_3$

FIG. 16

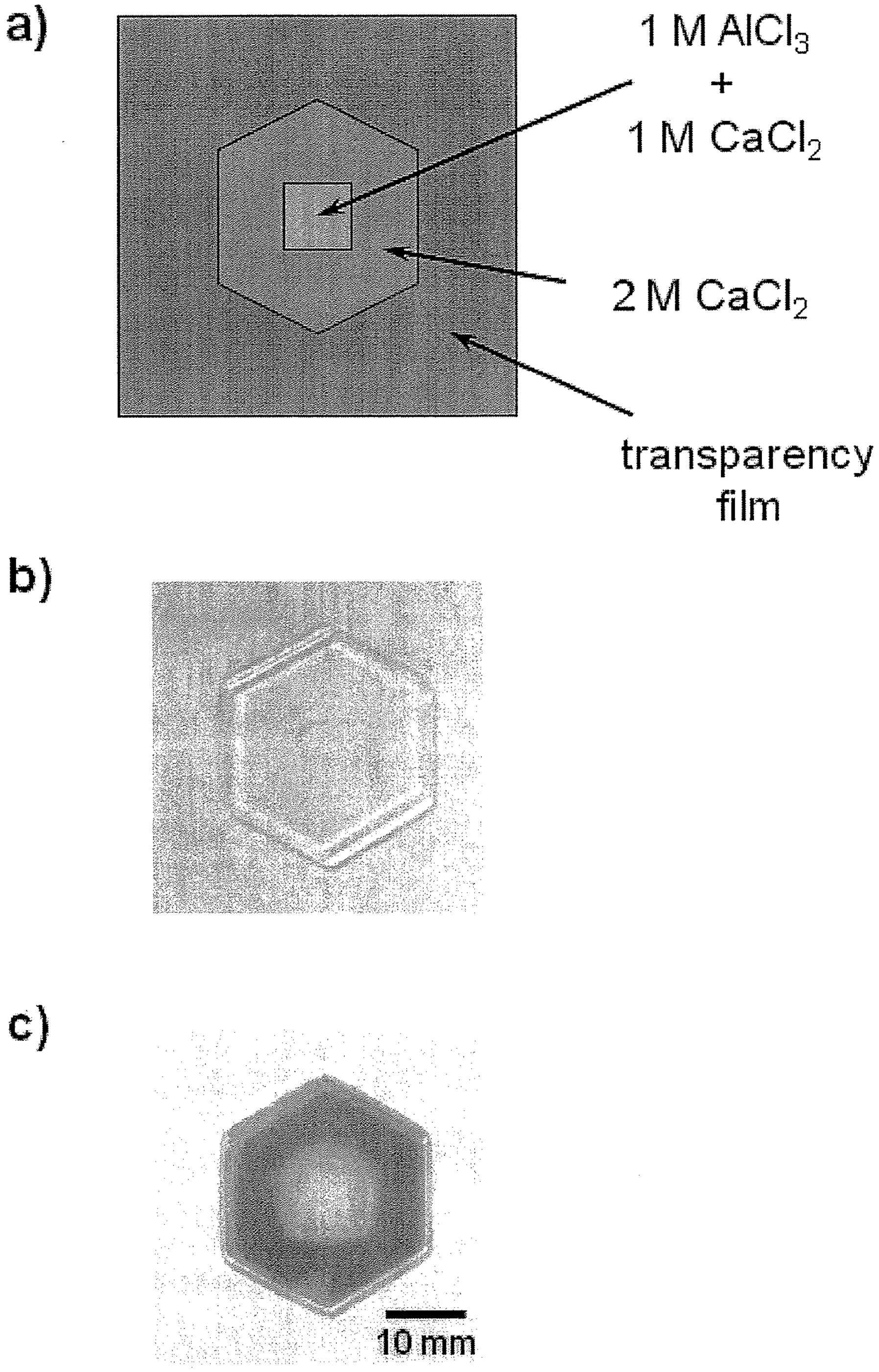


FIG. 17