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Photolithographically Assembled Polyelectrolyte Complexes as Shape-Directing Templates for Thermoreversible Gels

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ABSTRACT: Preparation of soft materials with diverse, customized shapes has been a topic of intense research interest. To this end, we have recently demonstrated photolithographic directed assembly as a strategy for customizing polyelectrolyte complex (PEC) shape. This process uses *in situ* photopolymerization of an anionic monomer in the presence of a cationic polymer, which drives localized PEC formation at the irradiation sites. Here, we show how such photolithographically assembled PECs can serve as structure-directing templates for tailoring the shapes of other soft materials; namely, thermoreversible gels. These templated hydrogels are prepared by adding a thermogelling polymer (agarose) to the anionic monomer/cationic polymer/photoinitiator precursor solutions so that, upon irradiation, custom-shaped PECs form within agarose gel matrices. Once these PECs are formed, the surrounding agarose gels are melted (through heating) and washed away which, upon returning the samples to room temperature, produces interpenetrating PEC/agarose gel networks with photopatterned shapes and dimensions. Dissolution of these sacrificial PEC templates in concentrated NaCl solutions then generates photolithographically templated agarose gels, whose shapes and dimensions match those of their PEC templates. Besides tuning their shapes and sizes, the mechanical properties of these gels can be easily tailored by varying the initial agarose concentrations used. Moreover, this PEC-templated gel synthesis appears to not adversely affect hydrogel cytocompatibility, suggesting its potential suitability for biological and biomedical applications. Though this study uses only agarose as the model gel system, this PEC-based strategy for customizing gel shape can likely also be applied to other thermoreversible gel networks (e.g., those based on methylcellulose, poloxamers or thermoresponsive chitosan derivatives) and could have many attractive applications, ranging from drug delivery and tissue engineering, to sensing and soft robotics.

Keywords: Photolithography, thermoreversible gels, polyelectrolyte complexes, hydrogels

1. Introduction

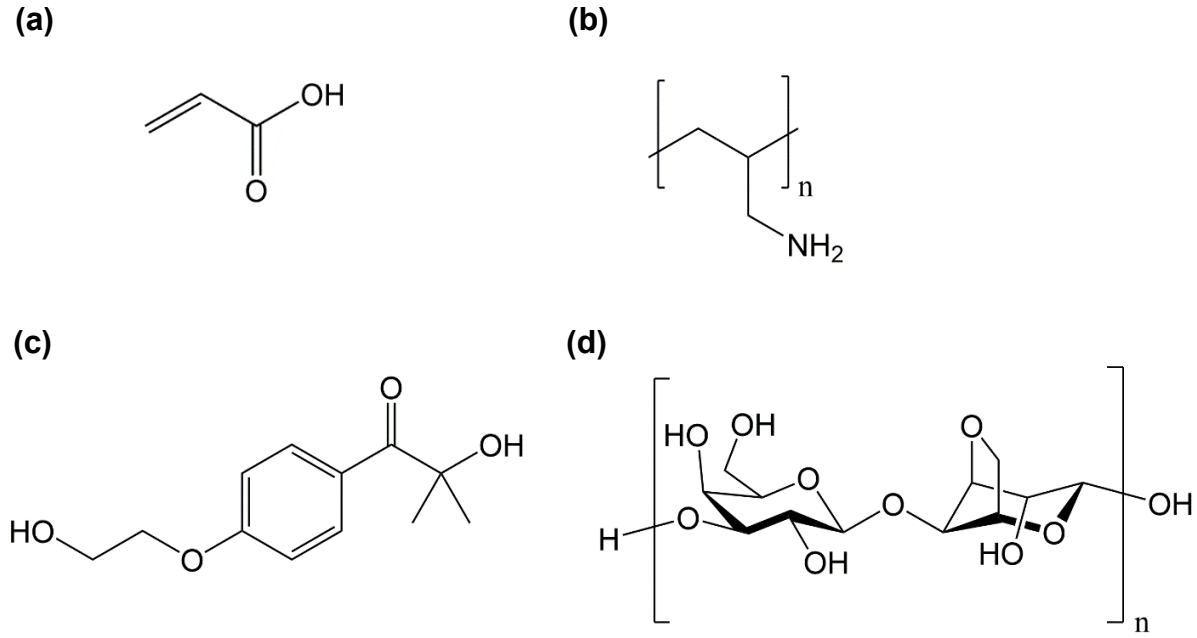
Polymeric hydrogels are water-swollen polymer networks that are crosslinked by either physical (ionic, hydrophobic or hydrogen) or chemical (covalent) bonds.[1,2] When prepared from biopolymers such as agarose, alginate and collagen, these materials are particularly attractive for pharmaceutical and medical applications,[3,4] due to their low toxicity,[5,6] tunable/stimulus-responsive properties[7,8] and biomimetic,[9] hydrated structures.[10,11] Aside from their biomedical use, polymeric gels are utilized in diverse fields such as bioseparations (adsorption,[12] chromatography[13] and electrophoresis[14,15]), biosensing,[16] enzyme immobilization,[17,18] and consumer products such as food packaging,[19] wound dressings[20] and liquid absorption;[21] as well as in more futuristic areas such as soft electronics and robotics.[22,23] Some of the above applications (e.g., tissue engineering, novel sensor development and soft robotics) require the preparation of hydrogels with application-specific, customized shapes and sizes.[24-26]

This shape and size control has traditionally been achieved by methods such as molding techniques (where polymer solutions are allowed to gel inside prefabricated, shape-directing molds),[27,28] electrospinning (to form cylindrical fibers)[29,30] and dropwise addition (where polymer solutions are added dropwise to a coagulating mixture to form spherical beads),[31,32] and cutting simple shapes out of gel sheets and slabs.[33,34] More recently, 3D printing has also emerged as a versatile way to control gel shape, where custom-shaped gel structures are generated through either direct ink writing (where gels are assembled through layer-by-layer extrusion of gelling polymeric solutions though micronozzles)[35-37] or stereolithography (where gelling monomer mixtures are locally polymerized by a scanning laser).[38,39] Customizing gel shape beyond simple spheres, cylinders and flat sheets with these techniques, however, requires access to advanced tools (such as 3D printers) or, in the case of molding techniques, custom-manufactured molds and, although 3D printing is becoming increasingly popular, these fabrication tools are not ubiquitously available.

An alternative strategy for customizing gel shapes, which (when the shapes required are not very complex) overcomes this need for custom-made molds or advanced instrumentation, is conventional photolithography.[40-42] In its simplest form, photolithography uses almost universally available supplies (i.e., a UV lamp and a homemade photomask), and such simple setups have enabled creation of an impressive range of micron- to centimeter-scale gel structures.[40,41,43-46] Chemically, this highly accessible photolithographic gel shaping approach has been achieved through either: (1) phototriggered photopolymerization/covalent crosslinking (which typically formed covalently crosslinked gels);[41,44-46] or (2) photoactivated changes in the ionic crosslinking and/or ionization (which occurred through either a phototriggered release of ionic crosslink-forming multivalent ions, or light-induced changes in protonation or oxidation states).[40,47-49] Such photolithographic shaping of gels generated through other chemistries, however – e.g., through supramolecular assembly of prepolymerized nonionic polymers, such as the widely used (hydrogen bonding) agarose[50,51] or (hydrophobically associating) methylcellulose and poloxamers[52,53] – has to our knowledge remained elusive.

To address this limitation of traditional photolithography, here we demonstrate the use of photolithographically assembled polyelectrolyte complexes (PECs) as templates for the shaping of nonionic, thermoreversible gels. We have recently shown how *in situ* photolithographic polymerization can be used to customize the shape of PECs, where the photopolymerization of an anionic monomer at the irradiation site leads to site-specific PEC formation.[54] To achieve this, acrylic acid (AA) was used as the monomeric anion, poly(allylamine) (PAH) was used as the polycation and Irgacure 2959 (IC) was used as the photoinitiator (see Scheme 1a – c). Upon polymerizing the AA to poly(acrylic acid) (PAA), PAH/PAA complexation rapidly generated PECs whose shapes could be tuned by controlling the photoirradiation pattern (using either a simple UV lamp and paper photomask or more sophisticated two-photon lithography).[54]

Scheme 1. Chemical Structures of (a) AA, (b) PAH, (c) IC 2959 and (d) Agarose.



Building on this idea, here we show how this photodirected PEC assembly can be harnessed to tune the shape of thermoreversible gels, using the biopolymer agarose (Scheme 1d), which gels at room temperature but melts when heated, as the model thermoreversibly gelling polymer. Specifically, we explore the possibility of creating custom-shaped hydrogels using photolithographically assembled PECs as structure directing templates. Similar to their parent PECs,[54] the custom-shaped hydrogels can be made to have submillimeter feature sizes. Besides demonstrating how to customize the gel size and shape, we show how the stiffness of these PEC-templated gels can be controlled by tuning the initial concentration of the gelling polymer, which enables the mechanical properties of these gels to be tailored to their end use. We also investigate the variation of the hydrogel size with irradiation time and probe the dimensional fidelity of the final gels to their photolithographically generated parent PECs. Finally, to start assessing the applicability of these gels in biological and biomedical applications, their cytocompatibility with model mammalian cells is also briefly explored.

2. Materials and Methods

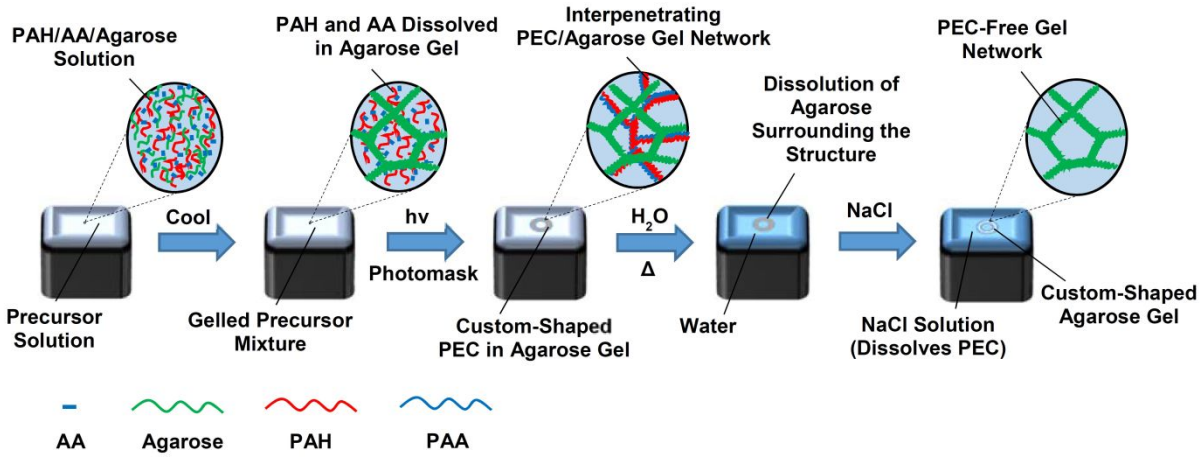
2.1 Materials. Deionized water with a 18.2 MΩ·cm resistivity was obtained from a Millipore Direct-Q water purification system and used in all experiments. AA served as the anionic monomer, while IC was the photoinitiator. Both AA and IC, along with agarose (Type I, low EEO), were obtained from Sigma-Aldrich (St. Louis, MO). The cationic polyelectrolyte PAH (nominal molecular weight of 15 kDa; sold in its deprotonated form as a 15 wt% solution) was purchased from Polysciences, Inc. (Warrington, PA). Methanol, hydrochloric acid, sodium chloride, sodium hydroxide, sodium phosphate tribasic dodecahydrate, HyClone™ Dulbecco's Modified Eagles Medium (DMEM), HyClone™ Calf Serum and Cell Titer 96® AQ_{ueous} One Solution were all obtained from Fisher Scientific (either Fair Lawn, NJ or Oakville, ON). All materials were used as received.

2.2 Preparation of Precursor Solutions. To prepare precursor solutions for the custom-shaped agarose gels, variously concentrated (1.4 – 5.6 wt%) agarose solutions were generated by dispersing 25 – 100 mg of agarose powder in 1.79 mL of water and heating the dispersions in an 80 °C water bath for 20 min while stirring at 120 rpm. In a separate vial, 0.34 mL of neat AA and 1.87 mL of 15 wt% aqueous PAH solution were mixed together. To add the photoinitiator, 11.3 mg IC was initially dissolved in 1 mL of methanol, whereupon the entire IC/methanol charge was added to the PAH/AA solution. The PAH/AA/IC solution was then added to the aqueous agarose solution and allowed to mix for an additional 10 min in the water bath to form 5 mL of a 1.0 M (5.8 wt%) PAH, 1.0 M (7.2 wt%) AA and 0.01 M (0.23 wt%) IC solution that contained 0.5 – 2.0 wt% agarose.

2.3 Preparation of Agarose Gels. To produce PEC-templated agarose gels, 1-mL precursor solution aliquots were deposited into chambered microscope slides (Lab-Tek® II Chambered #1.5 German Coverglass System). The solutions were then cooled, initially at room temperature for 20 min and then at 12 °C for 10 min, to turn the precursor solutions into gels (Scheme 2). Finally, the

gelled precursor mixtures were allowed to equilibrate at room temperature for an additional 6 min. The chambers were then covered with photomasks – which were prepared by cutting out different shapes in yellow 3M Post-It® Note paper and mounting the patterned paper onto Fisherbrand® microscope glass coverslips with 3M Permanent Double-Sided Scotch® tape – using the same double-sided tape to attach the glass coverslips to the tops of the chambers. The samples were then irradiated through their photomasks for 11.5 min with a UVP Blak-Rays B-100AP High Intensity UV lamp (λ = 365 nm, nominal intensity = 21 000 mW cm^{-2}), which was placed 10 cm away from the chambered microscope slide inside a black (3375 cm^3) cubic UV exposure box.

Scheme 2. Preparation of Agarose Gels.



The photopatterned agarose gel slabs (which now contained photolithographically assembled PEC templates) were then carefully lifted from their chambers, placed into 20 mL of water inside 40-mL glass scintillation vials, and heated in a Benchmark® MultiTherm thermostated shaker at a set-point temperature of 100 °C and an agitation speed of 200 rpm for 30 min. This heating/washing step melted and washed away the agarose surrounding the custom-shaped PECs (Scheme 2), but was not long enough to elute the agarose from inside the PEC templates (vide infra). Finally, the PECs were washed twice in 10 mL of room-temperature DI water (for 5 min each time) to remove any PEC debris that sometimes formed due to inadequate AA polymerization near the edges, and to allow the agarose gel networks inside the PECs to reform. The resulting

interpenetrating PEC/agarose networks were then washed in 3.5 M NaCl solutions for 2 d at a stirring speed of 150 rpm to dissolve and wash out the sacrificial PEC templates (see Scheme 2). This PEC dissolution method was selected over the use of concentrated acid and base solutions (which also dissolve PAH/PAA PECs by eliminating the PAA or PAH charges[54]) because it was a milder treatment – e.g., it did not subject the gel components to acid- or base-catalyzed hydrolysis. Indeed, when the 3.5 M NaCl wash was replaced with an overnight pH 1.5 HCl wash, the agarose gels crumbled into pieces. To prevent the samples from being struck by the 50.8 mm × 8 mm octagonal stir bar during the NaCl wash, they were placed on top of perforated aluminium foil partitions, which kept the custom-shaped structures away from the stir bar while ensuring sufficient convective mass transfer. After the salt wash, the ambient NaCl concentration was changed from 3.5 M to 0.15 M (i.e., to a near-physiological level) by gradual dilution with pH 7 sodium phosphate buffer (10 mM) to minimize the osmotic shock upon the ionic strength reduction. The final custom-shaped gels were imaged at 8× magnification using a Leica EZ4D (Buffalo Grove, IL) digital stereomicroscope and then stored in pH 7 phosphate buffer solution until further use.

2.4 Dynamic Rheology. The rheology of the PEC-templated agarose gels was investigated using a TA Instruments DHR-3 (New Castle, DE) strain-controlled rheometer, equipped with 8-mm parallel plates. Prior to loading, the gels were gently wiped using Kimwipes to remove any surface water. Upon loading, the gels were compressed to a 0.5-mm gap thickness, whereupon excess sample was gently removed from the sides of the plates with a spatula. The samples were then allowed to equilibrate for 60 s prior to each measurement. Strain amplitude sweeps were first performed to determine the linear viscoelastic region, following which frequency sweeps were performed at a strain amplitude of 0.1% and angular velocities (ω-values) of 0.1 – 100 rad/s. Each frequency sweep was conducted at 25 °C and repeated in triplicate.

2.5 Fidelity Studies. To examine the fidelity of the final gels to their PEC templates, photomasks with variably sized circular openings were generated by punching 0.7 – 6.3 mm diameter holes in

3M Post-It® Notes. Each note was then taped to a piece of polyester TruOffice transparency film using 3M Permanent Double Sided Scotch® tape. To form the PEC templates, 1 mL of precursor solution was deposited into a 2.7 cm × 2.7 cm × 0.1 cm petri-dish chamber created with Teflon gasket tape. To minimize optical distortions and oxygen-induced inhibition of polymerization kinetics, the photomasks were placed directly on top of precursor solutions (to ensure that there were no air gaps). Since there were no gaps between the precursor mixtures and the photomasks, the PECs (especially when they were small) would adhere to the glass coverslips when the photomasks were mounted on glass (see Section 2.3). Thus, the use of polyester TruOffice transparency film instead of the glass coverslips ensured that the complexes did not adhere to the masks (and could be easily removed from the chambers with minimal shape distortion). Once the photomask-covered sample chambers were formed, they were allowed to cool (first at room temperature for 20 min and then at 12 °C for 10 min) to gel the agarose, whereupon they were irradiated (as described in Section 2.3) for either 8, 10, 12 or 14 min (which allowed the effects of both photomask opening size and photoirradiation time on the fidelity of the templating process to be systematically explored).

Upon irradiation, the photopatterned PECs were carefully removed from the chambers and heated to melt the surrounding agarose. To prevent the gels from sticking to the surface of the glass vials during the heating/melting step, the smaller PECs (prepared using 0.7 and 1.7-mm photomask openings) were heated in 50 mL Thermo Scientific™ polypropylene centrifuge tubes instead of the 40 mL glass vials used for the larger structures. The final agarose gels were then obtained using the PEC dissolution procedure described in Section 2.3. The fidelity of the final agarose gels to their PEC templates was then investigated by comparing the diameters of the agarose gels to their parent PEC templates by imaging both with the Leica EZ4D digital stereomicroscope and measuring their dimensions using ImageJ (U.S. National Institutes of Health; Bethesda, MD). Each measurement was repeated using three replicate samples.

2.6. Cytocompatibility Analysis. To explore whether the custom-shaped gels were cytotoxic to mammalian cells, templated agarose rings (outer diameter 6.3 mm; inner diameter 3 mm) were

prepared as described in Section 2.3. Following the gradual dilution step, the agarose rings were stored in 10 mM phosphate buffer solution (at pH 7.0) until further use. Prior to their cytocompatibility analysis, the gels were sterilized in a 70:30 ethanol-water solution for 6 h and then soaked in sterile water overnight. To start the experiment, 3.0×10^4 murine NIH 3T3 fibroblast cells in 500 μL of growth media (10% fetal calf serum in DMEM) were seeded into a 24-well tissue culture plate and agarose rings were added to the wells in direct contact with the cells. The samples were incubated for 24 h at 37 °C and 5% CO_2.

To quantify the cell viability using a colorimetric assay technique, 100 μL of Cell Titer AQ_{ueous} One Solution (Promega Corporation; Madison, WI) reagent was added to each well and incubated for 1 h at 37 °C and 5% CO_2. The tetrazolium compound in the reagent was reduced by viable cells (in the presence of an electron coupling agent) to yield a water-soluble formazan product. This conversion was caused by metabolically active cells and the quantity of formazan was calculated by measuring the absorbance at 490 nm. Thus, the absorbance, which was read using a BioTek® Cytation 5 (Winooski, VT) plate reader, revealed cell viability. All absorbance values were normalized to cells grown in the absence of agarose gels.

Besides probing the cytocompatibility of PEC-templated agarose gels (with the sacrificial PEC templates removed as described in Section 2.3), the cytocompatibility of agarose gels still containing the PEC templates (which were not washed in 3.5 M NaCl solution) was also examined. Further, as an additional control, agarose gels prepared without the photopatterning procedure (i.e., by simply gelling the agarose solution as described in Section 2.3 and cutting the resulting gels into millimeter-scale rectangular pieces) were also tested for cytocompatibility. Each of these three sample types were prepared and tested at the highest (2.0 wt%) and lowest (0.5 wt%) agarose concentrations used in this study, using 4 replicates of each sample type. The mean absorbances from all samples were compared to the untreated (agarose-free) control and checked for statistical significance ($p < 0.05$) using a one way ANOVA and a Tukey post hoc test.

3. Results and Discussion

3.1. Preparation of PEC-Templated Agarose Gels. Upon irradiation of the PAH/AA/agarose mixtures through photomasks, custom-shaped PECs formed inside the agarose gels (Fig. 1a). Once the PEC-containing gels were melted through heating, free-standing PECs, whose shapes matched those of the photomask openings, were successfully isolated (Fig. 1b). Because these PECs also contained agarose, interpenetrating networks formed upon cooling of these PECs to room temperature, where agarose gels (which reformed within the PECs upon cooling) were intercalated within the PEC matrices as shown in Scheme 2. Finally, once the interpenetrating network structures were placed in 3.5 M NaCl solution, the opaque PECs rapidly dissolved (Fig. 1c), leaving behind translucent agarose gels. The dissolved PAH and PAA (along with the small-molecule impurities) then readily diffused away into the NaCl solution, leaving behind polyelectrolyte-free agarose gels (see ESI†, Section A). Using this approach, an array of diverse gel shapes (ranging from toroids, to flowers and party masks; Fig. 2) could be prepared without the use of customized molds[55,56] or advanced instrumentation,[35,38] which are frequently used for shaping thermoreversible hydrogels.

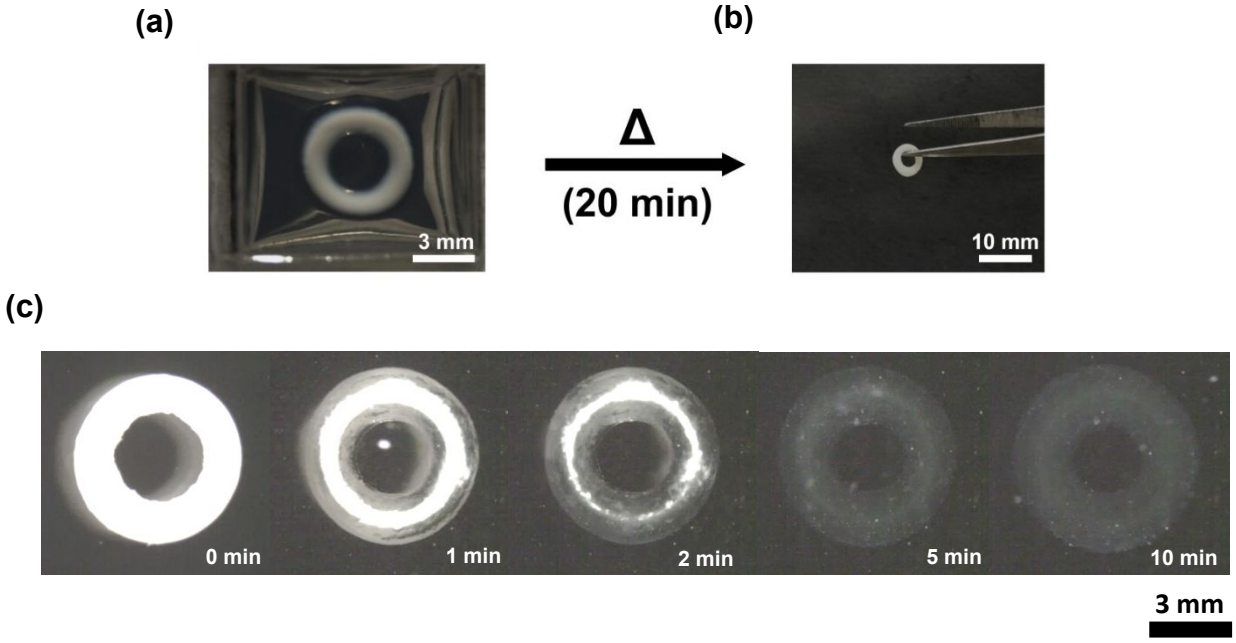


Fig. 1. Images of: (a) a PEC toroid formed within a 0.5 wt% agarose gel; the same toroid after (b) being isolated by melting and subsequently washing away the surrounding agarose; and (c) the PEC dissolution in 3.5 M NaCl solution, where the opaque PEC dissolves and leaves behind a translucent, PEC-templated agarose gel.

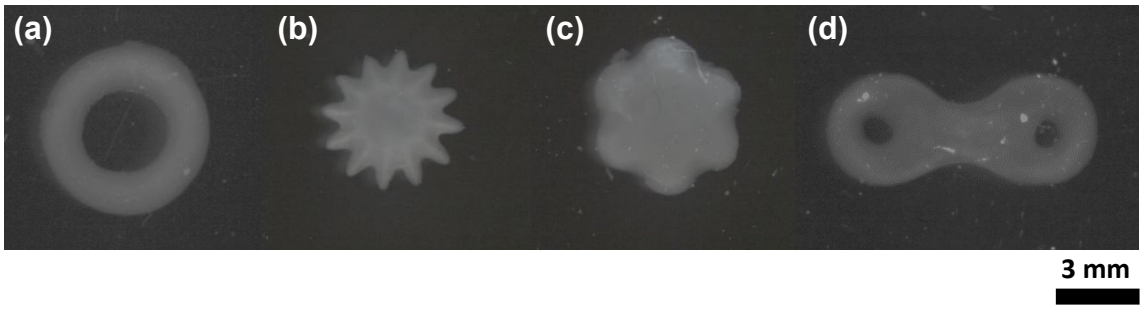


Fig. 2. Examples of PEC-templated agarose gels, shaped as (a) a toroid, (b) sun, (c) flower and (d) party mask.

3.2. Rheology of PEC-Templated Gels. To explore whether the PEC-templated gel stiffness could be tuned, their dynamic rheology was investigated. Similar to agarose gels prepared through traditional methods (i.e., through high-temperature agarose dissolution followed by cooling inside a mold), the storage moduli (*G'*-values) of these gels were both largely insensitive to the oscillation

frequency (ω) and consistently larger than the loss moduli (*G"*-values; see Figs. 3a, b and S2).[57] More importantly, the stiffness (*G'*) of these gels increased sharply with the agarose content in the parent PAH/AA/agarose mixtures, from 280 Pa for 0.5 wt% agarose to 3.3×10^4 Pa for 2.0 wt% agarose (when measured at ω =1 rad/s; see Fig. 3b and c), which again qualitatively agreed with prior findings on their traditionally prepared (non-templated) counterparts.[58,59] Further, the *G'*-values obtained for these PEC-templated agarose gels were quite similar to those of non-templated agarose gels with the same polymer contents and insensitive to the photoirradiation time; though templated gels prepared using 0.5 – 1.0 wt% agarose were slightly softer than their non-templated counterparts, while templated gels prepared using 1.5 – 2.0 wt% agarose were slightly stiffer (see Fig. 3d and ESI†, Section C). These modest differences in gel stiffness may have reflected PEC effects on the agarose gel structure (i.e., possible PEC effects on the agarose gel homogeneity and/or density of hydrogen bond crosslinks) or, in the case of the reduction in moduli upon PEC templating, some elution of the PEC-entrapped agarose during the heating step. Nonetheless, the close agreement between the *G'*-values of the PEC-templated and non-templated gels (and their strong sensitivity to the initial agarose content) indicate: (1) that the gels largely retain their moduli after the PEC-based templating procedure; and (2) that their mechanical properties can be extensively tuned by tailoring the agarose concentration in their parent PAH/AA/agarose mixtures.

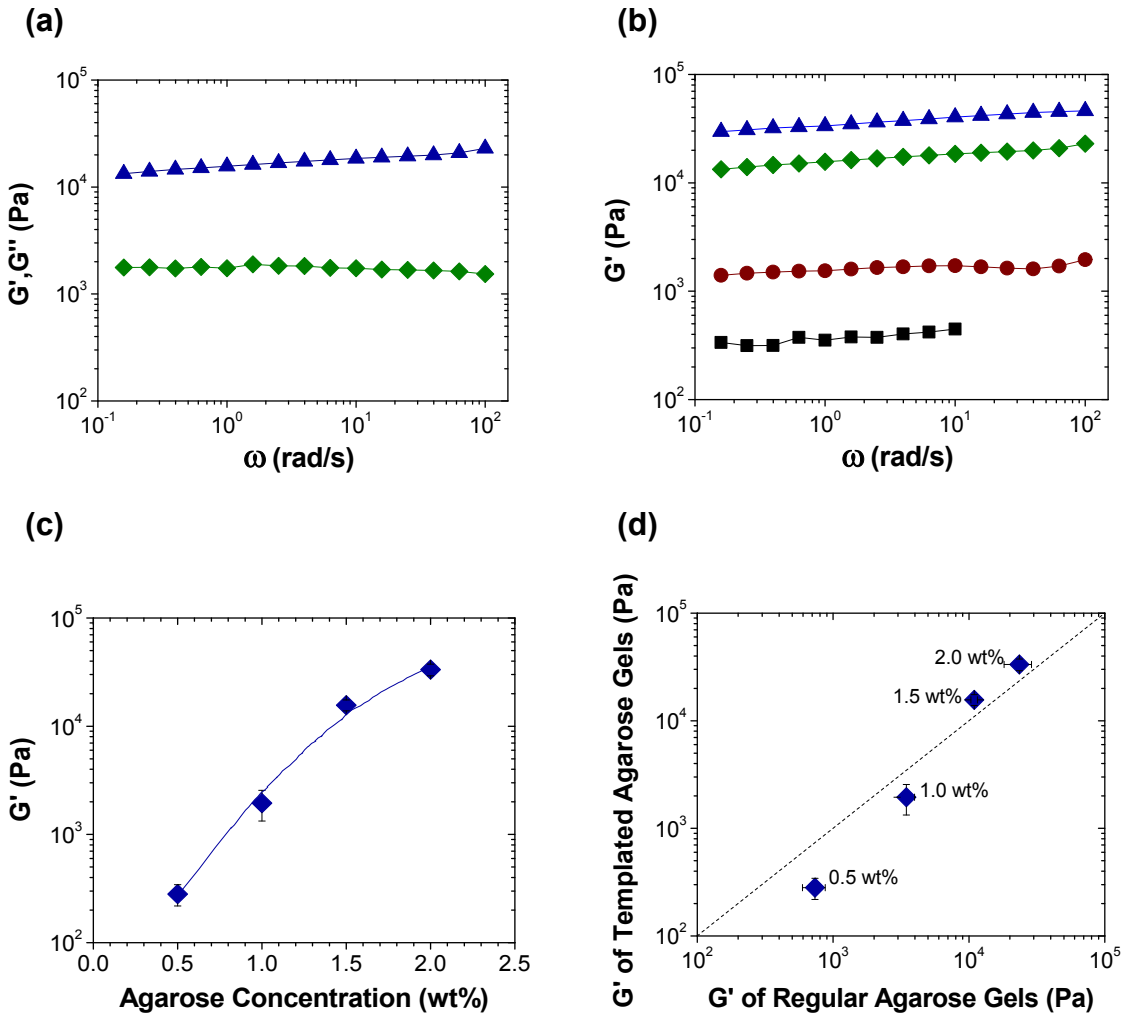


Fig. 3. Dynamic rheology of PEC-templated agarose gels showing: (a) representative data (obtained for gels prepared using 1.5 wt% agarose) comparing (▲) *G'* and (◆) *G''* as functions of ω; (b) *G'*-values as functions of ω for gels prepared using (■) 0.5 wt%, (●) 1.0 wt%, (◆) 1.5 wt% and (▲) 2.0 wt% agarose; (c) *G'*-values (at ω = 1 rad/s) plotted as a function of the agarose concentration used during photopatterning; and (d) a parity plot comparing *G'*-values (at ω = 1 rad/s) of PEC-templated agarose gels to those of regular (non-templated) gels prepared using 0.5 – 2.0 wt% agarose concentrations. The error bars are standard deviations while the lines are guides to the eye.

3.3. Fidelity Analysis. To examine the fidelity of the final gel dimensions to their shape-directing PEC templates, agarose gel discs were prepared using photomasks with four different millimeter and submillimeter-scale circular openings (Fig. 4a). As shown by the parity plot in Fig. 4b, the

diameters of the final gel discs were almost identical to those of their parent PECs across the entire investigated size range. Indeed, as illustrated in Fig. 4c, nearly all the final gel diameters were within roughly 10% of those of their parent PEC templates. These findings reveal fairly high fidelity between the final biopolymer gels and the parent PECs, indicating that (at least at the lengthscales targeted herein) substantial control over the gel size can be achieved by tailoring the irradiation pattern and time. Such dimensional similarity between the sacrificial PECs and their (final) agarose gel counterparts likely reflects two effects: first, that most of the agarose inside the parent PECs is not eluted during the heating/washing step (where the agarose surrounding the PECs is melted and washed away; Section 2.3); and second, that changes in the gel swelling upon PEC template removal are small.

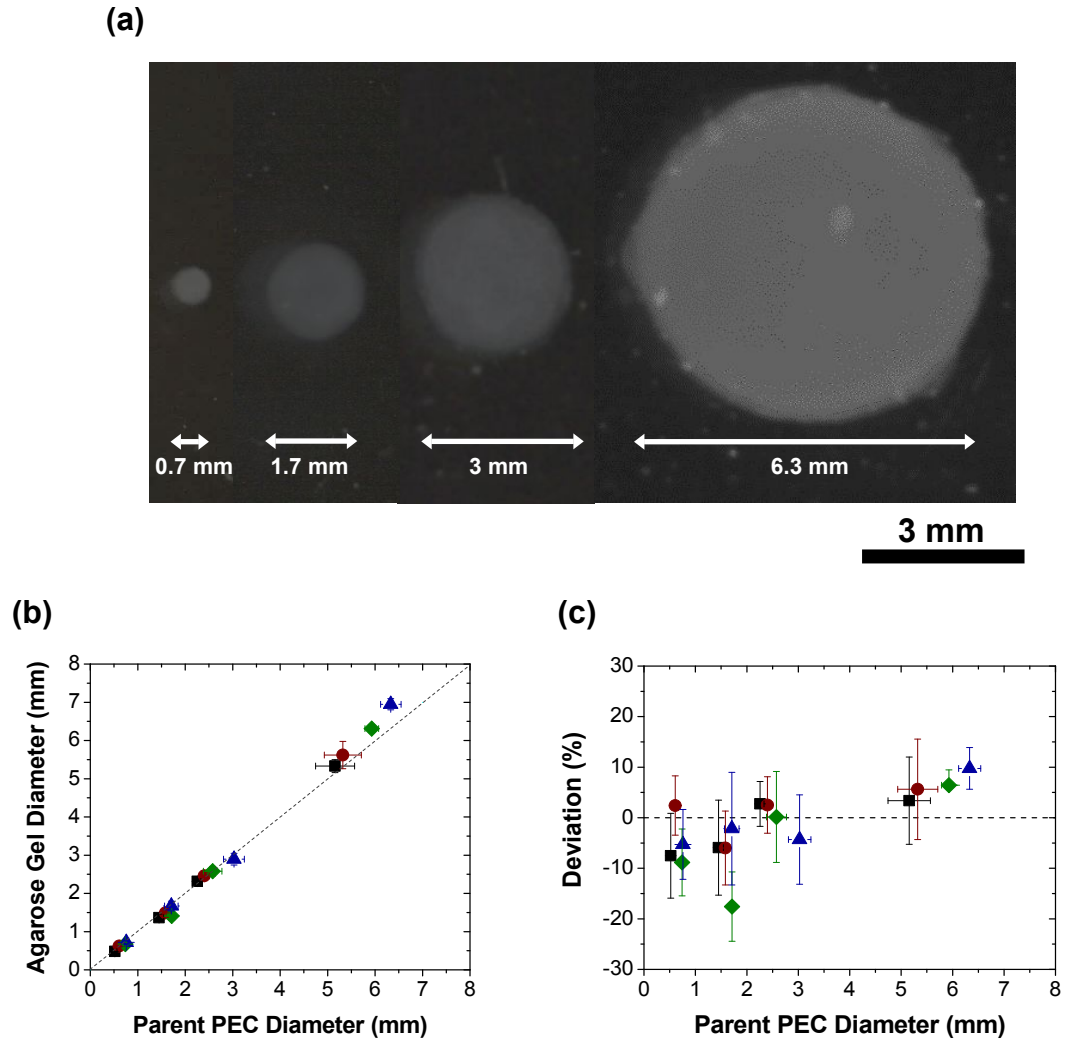


Fig. 4. Images of (a) disk-shaped agarose gels (0.5 wt%) prepared in four different sizes using photomasks with 0.7, 1.7, 3 and 6.3-mm circular openings; and comparison of agarose gel disk diameters to those of parent PECs prepared using (■) 8-min, (●) 10-min, (◆) 12-min and (▲) 14-min photoirradiation times via (b) a parity plot illustrating the correlation of the final agarose gel dimensions to those of the parent PECs; and (c) a plot indicating the percent deviation between the final agarose gel diameter and that of the parent PEC template (plotted as a function of the parent PEC diameter). The error bars are standard deviations and all experiments were performed using 0.5 wt% agarose.

Despite the high gel/template fidelity, there was an increase in the shape-directing PEC diameter with the irradiation time (see Fig. 4b), which occurred irrespective of the photomask opening size. The magnitude of this increase, however, varied with the photomask opening

diameter, ranging from 46% for the 0.7-mm photomask opening (where the average PEC diameter increased from 0.52 to 0.76 mm as the irradiation time was raised from 8 min to 14 min) to a 23% increase for the 6.3-mm photomask opening (where the average PEC diameter increased from 5.16 to 6.33 mm with the same change in irradiation time). For shorter (e.g., 8-min) irradiation times, the PEC diameters were consistently smaller than the photomask openings. Indeed, though insoluble PECs formed through the above photomasks were detected after roughly 5 – 6 min of irradiation, they were much smaller than the photoirradiated volumes, indicating that longer (≥ 8-min) irradiation times, such as those used in this experiment, were required to achieve the desired control over PEC size and shape. For the 14-min irradiation time, however, polyelectrolyte complexation (as indicated by the above PEC diameters) sometimes extended beyond the photoirradiation zone.

Because this time-dependent growth in PEC size was most pronounced at smaller photomask opening sizes, this effect imposed a lower limit on the sizes of the agarose gels that could be shaped with our simple photopatterning setup. When patterning with a photomask with 0.1-mm dimensions was attempted, for instance, the PECs almost immediately (within seconds of their initial formation) spread beyond the photoirradiation region. Such high sensitivity to photoirradiation time made it very difficult to pinpoint when to stop the irradiation with our setup (to ensure that the PEC template had the desired size and shape) and – though this limitation could likely be addressed through photochemical instrumentation upgrades – suggested that the simple photolithographic setup used herein is limited to custom-shaped gels larger than a few hundred microns in size.

Since the PEC formation rates depend on the AA polymerization kinetics, this variation in PEC size with the photoirradiation time also indicates that the AA polymerization kinetics are not spatially uniform. Such nonuniformity could stem from diffusion of the activated photoinitiator to regions beyond the irradiation zone. This diffusion could: (1) delay insoluble PEC formation near the edges of the photoirradiation site, which occurs at short irradiation times (likely due to a reduction in the local free radical concentration); and (2) ultimately cause PEC formation beyond the irradiation sites (which occurs at the longest irradiation time), since the diffusing radicals can

then polymerize the AA in the adjoining nonirradiated regions. Consistent with the above experiment, such diffusion effects are expected to be most impactful when the photomask openings are small.[60] Alternatively, since polymerization rates depend on the incident UV light intensity,[61] delays in PEC formation near the edges of the irradiation site may also partially reflect spatial gradients in the UV light intensity (where the intensity may slightly diminish with the distance from the center). Regardless of the reason for the temporal variation in the PEC size, however, the high fidelity between the final gels and their sacrificial PEC templates indicates that considerable control over the thermoreversible (agarose) gel dimensions can be achieved by simply tuning the shapes of their parent photolithographically assembled PECs.

3.4. Cytocompatibility Analysis. To start evaluating the suitability of PEC-templated gels for biological and biomedical applications, viability analyses with mammalian fibroblast cells were performed (see Fig. 5). Interestingly, regardless of whether the sacrificial PEC templates were washed out of the gels (cf. unwashed and washed groups), the cell viability was the same as in the gel-free media control wells. This result was achieved irrespective of the parent agarose concentration and indicated that, like regular (non-templated) agarose gels prepared through traditional methods – which were used here as another control and when unmodified are both non-cell-adhesive and noncytotoxic (see Fig. 5)[62,63] – these PEC-templated gels are compatible with mammalian cells.

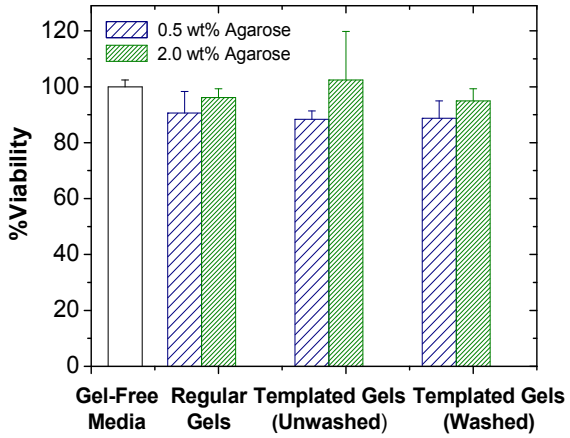


Fig. 5. Cell viability normalized to a "gel-free media" control. The error bars are standard deviations. Statistical analysis using a one-way ANOVA with Tukey's post hoc test revealed no significant differences between the groups ($p > 0.05$).

Also notable was the high viability achieved in unwashed gels (which still contained PECs) since, though the photoinitiator IC and PAA are known to be cytocompatible,[64-66] both PAH[67] and monomeric AA[68] can be cytotoxic. The free AA, however, was likely washed away during the washing/storage of the gels in saline (see Section 2.6). Conversely, the apparent absence of PAH toxicity likely reflected a lack of available (free) PAH amine groups due to PAH/PAA complexation. Similar complexation-mediated cytocompatibility has previously been shown upon the complexation of PAH with anionic dextran sulfate[69] and tripolyphosphate.[70] Additionally, the cytotoxic PAH functional groups may be further shielded from the cells by adsorbed growth media proteins and by the agarose, which may extend beyond the surface of the PEC. Though the relative contributions of these effects remain unknown, the promising cytocompatibility of the PEC-templated agarose gels suggests that PEC-templated hydrogels could potentially be used in biological and biomedical applications.

3.5. Further Discussion. Overall, the photolithographically assembled PEC templates enable a widely accessible and versatile strategy for shaping thermoreversible gels. This technique, which expands the range of hydrogel chemistries that can be made compatible with traditional

photolithography, enables the creation of custom-shaped gels with centimeter- to submillimeter-scale features and with tunable mechanical properties. Though here we only focus on gels based on the biopolymer agarose, this templating technique can likely be extended to other thermoreversible gels, such as those formed from thermogelling cellulose derivatives (e.g., methylcellulose and hydroxypropyl methylcellulose),[53,71] chitosan derivatives[72,73] and poloxamers.[52] Such custom-shaped gels could have diverse applications, ranging from novel sensors[74] to soft electronics and robotics.[22,23] Moreover, since the photolithographic PEC templating preserves the cytocompatibility of the gels, these custom-shaped gels could be utilized in biological and biomedical applications, such as tissue engineering[75,76] or microfluidic bioreactor design.[77]

It may also be possible to use the photolithographically assembled PEC templates for directing the shapes of other material types. Xu *et al.*, for instance, have generated PECs by direct ink writing (i.e., 3D printing through extrusion of liquid-phase polyelectrolyte mixtures through micronozzles into precipitating solvent mixtures) and used these custom-shaped PECs as synthesis templates for micropatterned ceramic (silica) structures.[78] Thus, the photolithographically assembled PECs utilized herein, may also provide an alternate route to shaping inorganic materials.

While our photolithographic PEC-templated gel shaping method can be combined with advanced microfabrication techniques (e.g., two-photon photolithography[54,79] and other photolithographic 3D printing technologies[80]), which could enable its extension to smaller and more intricate structures, in its simplest form it requires no specialized equipment other than a UV lamp and paper-based stencil mounted on a glass or plastic coverslip. Given the widespread availability of these supplies, this strategy to shaping thermoreversible gels (and possibly also other materials) could enable customs-shaped material/device fabrication in a broader range of research settings, and thus accelerate the emergence of new materials and technologies.

4. Conclusions

We have developed a novel, mold-free technique for preparing custom-shaped thermoreversible gels. This technique, which expands the range of materials that can be shaped through conventional photolithography, requires no special instrumentation other than a UV lamp and a paper-based photomask, and uses photolithographically assembled PECs as shape-directing sacrificial templates. Removal of the PEC templates enables the formation of custom-shaped gels of varying shapes and sizes, which span centimeter- to submillimeter-scale lengthscales and whose stiffness can be readily tuned by adjusting the gelling polymer concentration. The thermoreversible gel dimensions exhibit high fidelity to their PEC templates, indicating that the gel shapes can be reliably controlled by tailoring the shapes of their sacrificial templates. Moreover, the PEC-templated agarose gel synthesis has no impact on the gel cytocompatibility with model mammalian cells, which suggests that this photolithographic gel synthesis can be used for biological and biomedical applications. Though this study only used agarose as the thermoreversibly gelling polymer, this gel shaping strategy can likely be extended to shaping other types of thermoreversible hydrogels and, possibly, to even shaping other classes of materials (e.g., ceramics). Since this templating approach (in its simplest form) requires no specialized equipment other than a UV lamp, it could enable custom-shaped materials synthesis in a broader range of research laboratories and, accordingly, advance the development of new technologies in areas ranging from tissue engineering, to new sensors and soft robotics.

Conflicts of Interest. There are no conflicts to declare.

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Table of Contents Entry

Photolithographically prepared polyelectolyte complexes (PECs) can serve as shape-directing, sacrificial templates for nonionic thermoreversible gels.