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Fabrication of Functional Biomaterial Microstructures by in Situ Photopolymerization and Photodegradation

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Abstract

The in situ fabrication of poly(ethylene glycol) diacrylate (PEGDA) hydrogel microstructures within poly(dimethylsiloxane) (PDMS)-based microfluidic networks is a versatile technique that has enabled unique applications in biosensing, medical diagnostics, and the fundamental life sciences. Hydrogel structures have previously been patterned by the lithographic photopolymerization of PEGDA hydrogel forming solutions, a process that is confounded by oxygen-permeable PDMS. Here, we introduce an alternate PEG patterning technique that relies upon the optical sculpting of features by patterned light-induced erosion of photodegradable PEGDA deemed negative projection lithography. We quantitatively compared the hydrogel micropatterning fidelity of negative projection lithography to positive projection lithography, using traditional PEGDA photopolymerization, within PDMS devices. We found that the channel depth, the local oxygen atmosphere, and the UV exposure time dictated the size and resolution of hydrogel features formed using positive projection lithography. In contrast, negative projection lithography was observed to deliver high-resolution functional features with dimensions on the order of single micrometers enabled by its facilely controlled mechanism of feature formation that is insensitive to oxygen. Next, the utility of photodegradable PEGDA was further assessed by encapsulating or conjugating bioactive molecules within photodegradable PEG matrixes to provide a route to the formation of complex and dynamically reconfigurable chemical microenvironments. Finally, we demonstrated that negative projection lithography enabled photopatterning of multilayered microscale objects without the need for precise mask alignment. The described approach for photopatterning high-resolution photolabile hydrogel microstructures directly within

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ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge on the ACS Publications website at DOI: [10.1021/acsbiomaterials.8b00350](https://doi.org/10.1021/acsbiomaterials.8b00350).

Set of differential equations and variables used to compute COSMOL model for photopolymerization of PEGDA hydrogels using PPL (PDF)

The authors declare no competing financial interest.

PDMS microchannels could enable novel microsystems of increasing complexity and sophistication for a variety of clinical and biological applications.

Graphical Abstract

Keywords

microfluidics; biomaterials; projection lithography; hydrogel patterning

INTRODUCTION

The increasing adoption of lab-on-a-chip devices for clinical and biological applications has created a need for precise spatial, temporal, and geometric control over the composition and mechanical properties of miniaturized systems. Hydrogels have been utilized within microfluidic devices to establish control over biochemical microenvironments in space and in time for a variety of applications, owing to their versatility and biocompatibility, that allow the encapsulation of cells and proteins.^{1–6} For example, hydrogels formed by the photo-polymerization of poly(ethylene glycol) (PEG)-based mono-mers have been utilized to control stem cell fate, therapeutic delivery, and cell motility.^{7–12} Several methods have been introduced to create miniaturized hydrogel features, including micromolding or microcontact printing, contact lithography, laser lithography, and projection lithography.^{13–18} Combined with flow-based patterning of hydrogel composition, in situ hydrogel photolithography can be used to pattern hydrogel chemistry in addition to physical shape.^{19,20} All of these techniques provide different degrees of microscale control over hydrogel formation, chemical properties, and geometric resolution of microstructures on multiple length scales. However, only laser and projection lithography are noninvasive optical fabrication techniques that are capable of creating high-resolution hydrogel features within fully assembled micro-fluidic devices, giving the user on-demand control over the in situ microenvironment. In situ control of microfluidic microenvironments holds the potential to provide exquisite temporal, and potentially spatial, control over the mechanics, chemistry, presentation of biomolecules, and system geometry toward understanding cellular processes or creating bioassays within otherwise sealed, largely static microenvironments.

Commonly, microfluidic devices for biological applications are fabricated with poly(dimethylsiloxane) (PDMS), an inexpensive, biocompatible, and oxygen permeable

elastomer that allows the fabrication of devices that can sustain long-term cell growth.^{21–24} Within these devices, several approaches have been developed for the controlled presentation of biochemical cues with varied degrees of property control, including micromolding and microcontact printing, contact lithography, and projection lithography. Micromolding and microcontact printing typically employ patterned PDMS substrates to print or stamp patterns onto surfaces and have been utilized for a variety of applications, including patterned cell culture platforms, functional capture surfaces, and supported lipid bilayer membranes.^{25–30} While both techniques represent simple, multistep procedures that can be implemented in nearly any laboratory environment, their ease of use is counteracted by challenges with pattern reproducibility and device assembly after hydrogel layer development. Contact lithography is another easily implemented patterning technology that is accomplished by passing UV light through a shadow mask placed in contact with the surface to be modified. This method has been utilized to pattern the surface of glass and hydrogels, and by controlling the macromolecular constituents of the hydrogel solution, this approach has been applied to a wide range of biological and sensing applications.^{14,15,31–43} While contact lithography is a robust method for basic hydrogel patterning, it is limited in the feature size and resolution that can be achieved within PDMS microchannels due to the lack of optical mask reduction and separation between the mask and patterning substrate by a coverslip or slide. Additionally, multilayered hydrogels that can be used for dynamic microenvironment alterations are challenging to fabricate by contact photolithography as multiple flushing, precise alignment, and exposure steps are required. Projection lithography utilizing a subtractive fabrication method has the potential to decrease the complexity in forming multilayered features, thus creating opportunities for enhanced system intricacy.

Projection lithography (PL) is a robust technique that has been developed to allow for feature fabrication within sealed microfluidic devices by passing UV radiation through a shadow mask placed in the conjugate focal plane of an inverted microscope to polymerize and pattern hydrogel structures within microfluidic channels. This technique increases optical intensity, reduces exposure time, and reduces feature size relative to flood irradiation through a photomask placed on the surface of the hydrogel as in contact lithography. The resolution and size of features produced by PL is ultimately bounded by radical diffusion and oxygen inhibition. While generally considered a hindrance, controlled oxygen inhibition has been demonstrated to be enabling in creating micro-particles via continuous or stop flow lithography that can be used for various drug delivery and biosensing applications.^{13,19,44–49} However, a drawback to microscale photo-lithographic patterning by oxygen-inhibited photopolymerization remains the patterning of hydrogels within PDMS-confining environments and its deleterious effects upon cells, which can result in diminished cell viability.^{50–52} To overcome the limitations associated with photopolymerization of hydrogels by PL, we introduce a subtractive manufacturing technique by which large PEGDA hydrogels are photo-polymerized and individual features are subsequently sculpted by photodegradation. Subtractive hydrogel formation is independent of oxygen concentration, which we predict would allow for the formation of well-resolved structures in the presence of cells or biomolecules because the *o*-nitrobenzyl cleavage products have been shown to be nontoxic to various cell types (e.g., fibroblasts, human mesenchymal stem cells, and primary cells).^{6,53,54}

Here, we compared two PL techniques, positive projection lithography (PPL) and negative projection lithography (NPL), that circumvent the poor resolution, need for precise mask alignment, and chemical side effects of other photopolymerization-based in situ lithography methods for hydrogel fabrication. We quantified the challenges to control the size and resolution of in situ photopolymerized poly(ethylene glycol) diacrylate (PEGDA) hydrogels in both oxygen inhibited and nitrogen purged (oxygen eliminated) conditions. We next demonstrated an alternative lithography approach that utilizes photo-degradation, a process not affected by oxygen inhibition, via the optical cleavage of *o*-nitrobenzyl-containing PEGDA hydrogels. NPL is the photolithographic patterning of hydrogels within a microfluidic device by photodegradation, which enables the fabrication of high resolution and complex multilayer features. Hydrogel patterning by photopolymerization and photodegradation, analogous to negative and positive photoresists, respectively, were compared by demonstrating and quantifying the relative capabilities, merits, and challenges of both PL approaches. Finally, the ability to dynamically reconfigure biomicrofluidic systems with biomolecule-loaded photodegradable microstructures was demonstrated. Through noninvasive actuation with UV light, dynamic protein gradients were created within a sealed microfluidic environment. By utilizing both PL approaches presented here, innovative applications in tissue engineering, applied biology systems, and on-chip assays could be enabled.

MATERIALS AND METHODS

Device Fabrication and Surface Functionalization.

PDMS (Sylgard 184, Dow Corning) devices were replicated from photo-lithographically patterned silicon wafers using conventional soft lithography techniques.²³ PEG features were created within PDMS straight channels (5 cm in length and 4 mm in width) with depths of 20 or 50 μm . The thickness of the PDMS devices was approximately 10–20 mm for purged devices and 50–60 mm for ambient devices. Oxygen plasma treated PDMS channel replicas were bonded to plasma-cleaned glass coverslips.⁵⁵ Bonded channels were used for all PPL experiments. For the purged devices, an additional PDMS straight channel was fabricated that was 50 μm in depth and approximately two-thirds the length of the initial channel. This channel was bonded to the top of thin PDMS devices and used to flow nitrogen through to purge the device of oxygen. For NPL, acrylate-modified glass coverslips were used. Briefly, glass coverslips were cleaned using a Bunsen burner and placed in a solution containing 190 proof ethanol (Sigma-Aldrich) and 3-(acryloyloxy)-propyltrimethoxysilane (APTS, Alfa Aesar). The glass coverslips were removed after 5 min, rinsed with 190 proof ethanol, and placed in an oven to dry at 70 °C for a minimum of 20 min. PDMS channels were placed in contact with the acrylate-modified coverslips to form a spontaneous, reversible seal that was sufficient to maintain a bond during fluid exchange. Shadow masks used for PL were created in AutoCAD and printed as transparency masks (CAD Art).

Synthesis of Photodegradable PEGDA (PEGdiPDA).

An *o*-nitrobenzyl acrylate moiety was installed onto PEG-*bis*-amine ($M_n \sim 3400$ Da, Laysan Bio) using a previously described protocol.⁶ Briefly, acetovanillone was used to

synthesize 4-(4-(1-(acryloyloxy)ethyl)-2-methoxy-5-nitrophenoxy)butanoic acid (*o*-NB acrylate) through a multistep procedure.⁵⁶ The *o*-NB acrylate (4.4 equiv) was coupled to PEG-bis-amine (1 equiv) using carbodiimide chemistry with carboxylic acid activation using 1-[bis(dimethylamino)methylene]-1*H*-1,2,3-triazolo[4,5-*b*]pyridinium3-oxide hexafluorophosphate (HATU, 4.4 equiv) in the presence of *N,N*-diisopropylethylamine (DIPEA, 8 equiv). The reaction was completed overnight under argon gas at room temperature. The resulting functionalized polymer was precipitated into ethyl ether and obtained through centrifugation. The polymer was purified by dialysis against DI water (MWCO 1000 Da) and lyophilized to obtain an orange solid. The photocleavable polymer product, PEGdiPDA, was characterized by ¹H NMR spectroscopy (Bruker Daltonics, 600 Hz, 128 scans, deuterated DMSO) using the protons associated with the acrylate (6.35 ppm) and the amide (7.91 ppm) relative to the PEG backbone (3.5 ppm).

Synthesis of LAP.

The photoinitiator lithium phenyl-2,4,6-trimethylbenzolphosphinate (LAP) was synthesized based on a previously published protocol.^{57,58} Briefly, 2,4,6-trimethylbenzoyl (1 equiv) was slowly added to dimethylphenylphosphonite (1 equiv) under argon gas at room temperature. The mixture was left to react for 18 h, and then lithium bromide (4 equiv) in 2-butanone (100 mL) was added. The solution was heated to 50 °C until a solid precipitate formed. After precipitate formation, the reaction was cooled to room temperature and subsequently filtered. The filtrate was rinsed 3 times with 2-butanone, and excess solvent was removed under pressure. The dried product, LAP, was characterized using ¹H NMR (Bruker Daltonics, 600 Hz, 128 scans, CDCl₃).

Positive Projection Lithography.

A positive shadow mask was attached to the iris in the field aperture of an inverted microscope (Olympus IX81). The inverted microscope was fitted with a Prior Lumen 200 light source via a liquid light guide. Once the shadow mask was in place, a microfluidic channel was filled with PEGDA hydrogel forming monomer solution and placed on the microscope. The PEGDA hydrogel forming monomer solution was 60% w/w PEGDA (MW = 700 Da, Sigma-Aldrich), 1% w/w LAP, 1% v/v 1-vinyl-2-pyrrolidinone (NVP, Sigma-Aldrich), and 1% v/v acryloyloxyethyl thiocarbamoyl Rhodamine B (Rhodamine, Polysciences). Rhodamine B was used to facilitate focusing for photopolymerization on the microscope. The shadow mask was focused by excitation of Rhodamine B at a wavelength of 550 nm with a Texas Red filter cube (Semrock). The gel was polymerized using long wavelength UV light (DAPI filter with peak at $\lambda = 365$ nm, Semrock) with exposure times of 50, 100, 250, and 500 ms. MetaMorph Microscopy Automation and Image Analysis Software were used to control the exposure time through an automated shutter (Ludl).

Before each experiment was conducted, a test hydrogel was made to adjust for changes in bulb light intensity due to usage. For each test experiment, the microfluidic channel was filled with the hydrogel forming solution, the aperture of the microscope closed, and the sample was exposed to 365 nm light for the exposure times stated above. The microfluidic channel was flushed with PBS, and the hydrogels were imaged and compared to hydrogels formed on previous days. If hydrogels were not observed or not consistent, the light intensity

was adjusted by 1% increments until consistent hydrogel formation was observed. This process was also conducted on several different microscopes to adjust light intensity settings for consistent hydrogel formation.

Three different polymerization conditions were used to form features: an ambient microchannel with features formed under the 20× (NA = 0.45, $I_0 = 407 \text{ mW cm}^{-2}$) objective (ambient 20×), an ambient microchannel with features formed under the 40× (NA = 0.65, $I_0 = 383 \text{ mW cm}^{-2}$) objective (ambient 40×), and a nitrogen purged microchannel with features formed under the 20× objective (purged). For nitrogen-purged devices, the monomer filled channel was purged with nitrogen for 15 min before starting polymerization and continuously throughout the polymerization.

Negative Projection Lithography.

A microfluidic channel was filled with PEGdiPDA hydrogel forming monomer solution and placed on the microscope. The hydrogel forming monomer solution was 8.2% w/w PEGdiPDA, 1% w/w LAP, and 1% v/v Rhodamine, which was included for aiding in focusing of the shadow mask. A large post structure was polymerized within the microfluidic channel using the 20× objective (NA = 0.45) with the field aperture iris opened to create an exposed region of approximately 700 μm . These regions were created to minimize the amount of hydrogel degradation required to pattern isolated hydrogel features. Photopolymerization was achieved by irradiation of focused light passed through a 405 nm long pass filter (LP405, Olympus, $I_0 = 355 \text{ mW cm}^{-2}$) for 300 ms. After post polymerization, the channel was flushed with PBS to remove any unreacted monomer solution. A negative shadow mask was then placed in the field aperture of the inverted microscope and focused as described above. Once the shadow mask was focused, long wavelength UV light (365 nm, $I_0 = 407 \text{ mW cm}^{-2}$) was used to degrade the post structure leaving behind the desired features. Degradation exposure times of 2500, 5000, and 7500 ms were used.

Image Analysis.

The area and shape factor of the microstructures were determined using ImageJ.⁵⁹ The bright field image was converted to a binary image, and the “Analyze particles” feature was used to give the measured data. The two-dimensional area of the features was calculated using the height and width data obtained from the program. The circularity, a measure of how close the object shape is to a perfect circle, was used as reported in the software. Three of each geometry, at each exposure time and polymerization condition, were analyzed.

In Situ Hydrogel Degradation.

PEGdiPDA posts with Rhodamine copolymerized within were fabricated using visible light as described above. In this case, the incorporation of Rhodamine B was used to monitor hydrogel degradation. After post formation, any remaining monomer solution was flushed from the device with buffer. Posts were degraded by exposure to long wavelength UV light for 30 s. The diffusion of Rhodamine into the surrounding fluid was monitored as a measure of the change in the surrounding microenvironment by acquiring a fluorescent image every 30 s for 10 min. Image acquisition was controlled by MetaMorph, and the shutter was closed

between time points to avoid photo bleaching of the sample. Acquired images were analyzed using the Radial Profile plugin for ImageJ.

Multilayered Features.

Photodegradable posts were formed as described above except nonbonded microfluidic channels were used. The first layer was formed in a channel of 20 μm depth with a PEGdiPDA solution containing Rhodamine. After forming the first layer of posts, the channel was removed, and the posts flushed with PBS. A channel of 50 μm depth was placed over the top of the posts and filled with a PEGdiPDA solution containing AlexaFluor 488 (BSA-488, Invitrogen) at 20% v/v in place of Rhodamine. Rhodamine-containing posts were located; the aperture coarsely aligned with the post, and the second layer was polymerized on top of it using the same aperture size and an exposure time of 600 ms. Fine alignment was not required as nonoverlapping edges contributed a fraction of the available structure and could be avoided during final feature formation. The channel was flushed with PBS, and the posts were subsequently degraded by light irradiation passed through a shadow mask to form microstructures using the same procedure described above. Posts were imaged using a Zeiss laser scanning confocal microscope (Zeiss LSM 710) and stitched together using the ImageJ “Volume Viewer” plugin.

Photopolymerization Model.

To investigate the effects of oxygen inhibition and radical diffusion on PEGDA photopolymerization, a COMSOL reaction-diffusion model was used to simulate the in situ photopolymerization of PEG hydrogels containing 1% photo-initiator (LAP) over a range of exposure times. A free radical polymerization reaction step sequence was used in our model.^{51,60,61} Briefly, exposure to UV light causes photolysis of the photoinitiator (PI), which generates a radical species that triggers either chain initiation or chain propagation. The growing polymer chains are terminated by reacting with either another polymer chain or soluble oxygen.

The model used characterized two distinct but interacting reaction zones: the region in which radicals are formed by exposure to UV light (region 1) and the region outside the exposure area into which radicals migrate by diffusion (region 2). The first region was defined from the center of the projected cylinder ($r = -12.5 \mu\text{m}$) to its edge ($r = 0 \mu\text{m}$) and the second region was defined from r to infinity. The model considered the radial diffusion of monomer, PI, oxygen, and radical species throughout the defined region boundaries. A complete reaction sequence and the ordinary differential equations describing the reactant and product species balance can be found in the Supporting Information.⁶⁰

The model was simplified via assumptions based upon experimental observation and known experimental values. The UV intensity was considered to be constant throughout the channel depth, a reasonable assumption given the high optical intensity and shallow ($<100 \mu\text{m}$) channel depth used. A constant two-dimensional exposure projection was assumed for all z -positions in the channel. These assumptions establish a homogeneous polymerization initiation region throughout the channel depth and within the irradiated region. It was also assumed that the monomer, PI, oxygen, and radical species could diffuse from region 1 to

region 2, producing heterogeneous polymerization rates throughout the considered area. Further, it was assumed that there was no UV light exposure or radical generation from PI photolysis in region 2. This assumption ensured that any radicals, and thus polymerization, observed in region 2 were solely due to the diffusion of radicals from region 1. The diffusivity of radicals was assumed to be close to that of the PI, but its dispersion was limited by the fast conversion of radicals by chain growth, termination, or inhibition reactions. The conversion rate of monomer was assumed to be higher than that previously reported for Irgacure 2959 because experimental observations indicate that LAP polymerizes hydrogels approximately four times quicker than traditional PIs (such as Irgacure 2959).^{62,63} A complete list of the values used in the model can be found in the Supporting Information.

RESULTS AND DISCUSSION

Effect of Light Irradiation Time on the Size of Features Formed by PL.

Two PL techniques, positive and negative, were successfully demonstrated (Figure 1). Here, positive PL (PPL) was defined as the direct photopolymerization of PEGDA features (Figures 1a and c), and negative PL (NPL) was defined as the patterning *o*-nitrobenzyl (NB) containing PEGDA hydrogels by photodegradation (Figures 1b and d). To characterize and quantify the performance of these two biomaterial photopatterning motifs, three different conditions were assessed: (1) ambient conditions with PPL (ambient), (2) nitrogen purged conditions with PPL (purged), and (3) ambient conditions with NPL (negative). Nitrogen purging of the PDMS channel was utilized to eliminate excess oxygen and therefore the inhibitory effects of oxygen on the free radical initiated polymerization reaction scheme.⁶⁰ For all three photolithographic variations, the effect of UV irradiation time on feature size was characterized using the two-dimensional surface area of the formed post structures.

First, the formation of simple square features in a 20 μm deep PDMS channel with a 20 $\times$ objective (NA = 0.45) was investigated. The influence of UV irradiation was investigated by forming square features with an area of 775 μm^2 (27.8 $\mu\text{m} \times 27.8 \mu\text{m}$) or 22 μm^2 (4.69 $\mu\text{m} \times 4.69 \mu\text{m}$). For PPL conditions, exposure times of 50–500 ms were investigated. Consistent trends in feature size were established for features formed using PPL (Figures 2a–d). All features, under both ambient and purged conditions, were significantly larger than the projected mask area and feature size increased with increasing exposure time. The kinetics of photodegradation are slower than the kinetics of photopolymerization so the exposure times investigated for the NPL condition were increased to 2500–7500 ms. The size of features formed using NPL decreased with increasing UV exposure time and for all degradation times the features were smaller than the masked area (Figures 2a, b, and e).

When comparing PPL to NPL, the opposite trends in size of features formed were observed. For both PPL conditions, the features formed were statistically larger than the projected mask area. In contrast, NPL produced features that were smaller than the shadowed mask area. In general, these data indicate that higher resolution features with dimensions much closer to the mask area can be achieved using NPL rather than PPL. To quantitatively compare these techniques across all exposure times, the average difference in side length of the formed features and the mask was measured for both feature sizes investigated. Ambient PPL features were approximately $6.3 \pm 1.1 \mu\text{m}$ larger in side length than the mask, purged

PPL features $5.88 \pm 1.98 \mu\text{m}$ larger, and NPL features $0.49 \pm 0.74 \mu\text{m}$ smaller. It is of note in the PPL conditions that the change in area between the two mask sizes investigated was different, but the increase in linear feature size was similar, within error. This result confirms that feature size and resolution is limited by the diffusion of radicals into the surrounding media, which is independent of feature size. This mechanism is not observed during NPL feature formation, illustrating the unique capability to form smaller, higher resolution features via NPL.

In this particular demonstration, NPL produced features were on average $3.3 \pm 1.4\%$ smaller than the mask shadowed length, whereas, ambient and purged PPL features were on average 24.4 ± 5.8 and $22.1 \pm 7.7\%$ larger than the mask projected length, respectively (Figure 3a). Due to the different feature formation mechanisms, different trends in the data were observed, with a shrinking effect in NPL caused by the diffraction of light around the mask edges and an expanding effect in PPL caused by the diffusion of radicals into the surrounding media. These effects are exaggerated in both these cases with increasing UV exposure time. In addition to larger deviations between structure size and the mask size, larger variances were observed for PPL than NPL. This trend was absent in NPL formed features, where features are consistently coupled to mask size.

For PPL, the increase of feature size with increasing UV exposure time was hypothesized to be the result of radical diffusion radially into the surrounding hydrogel forming monomer solution. This hypothesis was tested using a two-dimensional reaction-diffusion model created in COMSOL Multiphysics software. Figure 3b plots the model-predicted extent of double bond conversion as a function of radial position. Time ranges from 0 to 1000 ms and increases by 100 ms time steps in order to approximate experimental conditions. Employing a commonly accepted relative conversion that defines hydrogel gelation, our model indicated that incomplete polymerization of the features persists until approximately 300 ms (see Supporting Information for more information).^{60,62} This is contradictory with our results which showed hydrogel formation at exposure times as low as 50 ms. This discrepancy in monomer conversion rate is most likely due to an underestimation of the activity of the PI, LAP, and the addition of NVP, an accelerant used to increase the monomer conversion rate.⁶³ However, the model did indicate that with increasing long wavelength UV exposure time, the double bond conversion fraction increases both inside the mask aperture as well as outside the mask projected radius, consistent with our experimental results. Modeling results also predicted incomplete gelation of the hydrogel solution beyond the mask projection radius at all time points, indicating that radical diffusion into the surrounding hydrogel forming solution alone explains the increase in feature size with increasing exposure time. These results also support our empirical observation that the growth of polymerizing features is uniform in linear distance and independent of mask size.

A second observation of note is that features formed by PPL within ambient versus purged devices, but otherwise identical exposure conditions, possess different equilibrium sizes. Features formed under these two conditions were similar in size (not statistically different) at short exposure times, but the features formed in purged microchannels were more rounded than those formed in ambient microchannels (Figures 2c and d). The balance between generation and consumption of radicals is different between the ambient and purged

systems, due to the reduction of oxygen in purged channels versus ambient channels. Initiator and monomer radicals react with oxygen while diffusing through an ambient PDMS micro-channel and can therefore no longer participate in polymerization. In nitrogen purged microchannels, conversely, oxygen is mitigated, leading to the preservation of higher PI and monomer radical concentrations, steeper radical concentration gradients, and therefore higher polymerization rates at increasing radial positions. The diffusion of radicals outward from the exposure source quickly becomes radially isotropic, resulting in rounded features and a loss of lithographic resolution. As expected, increasing UV exposure time increases feature size more significantly in purged PPL, but less so under ambient PPL conditions. Despite the loss of resolution, nitrogen purging is nevertheless useful as it facilitates the formation of hydrogel features that span the entire channel depth, which is prohibited in ambient microchannels due to an oxygen inhibition layer at the PDMS–monomer solution interface.^{62,64} The ability to form sample spanning hydrogel posts within PDMS microchannels offers a capability useful for biosensors and cell encapsulation that previously required oxygen impermeable glass or plastic microchannels.^{51,61,65}

In contrasting PPL with NPL, NPL clearly provides dramatically improved control over feature size and resolution. The size of features formed under NPL conditions were much closer to the mask dimensions and were not subject to a loss of resolution by diffusive broadening. Cleavage of the *o*-NB groups in the photodegradable PEG hydrogel post upon long wavelength UV irradiation dictates feature formation when using NPL; therefore, any feature size greater than the projected mask area is the result of incomplete hydrogel degradation. As the exposure time for degradation was increased, features smaller than the mask formed due to the undercutting of the mask by optical diffraction. This distinction highlights the different mechanisms by which features are formed via PPL versus NPL. In NPL, the influence of reaction kinetics and radical transport on microscale hydrogel formation is eliminated, which allows for complicated features of a desired shape, size, and spacing to be more readily fabricated. Features formed by NPL may be delicately tuned by adjusting the UV exposure time. Exposure times between 2.5 and 5 s, (Figure 3a) provide good fabrication conditions, as evidenced by the feature size area difference intersecting the mask area.

Formation of Complex Geometric Features Using PL.

The ability to lithographically pattern complex geometric features with high resolution and replication fidelity was investigated by forming star-shaped features under four different polymerization conditions. The three conditions (ambient PPL, purged PPL, and NPL with features formed using a 20× objective) described above were examined; in addition, features were polymerized under the 40× objective (NA = 0.65) using PPL in an ambient microchannel. A channel depth of 50 μm and a polymerization time of 100 ms was used for all PPL conditions and a channel depth of 20 μm and a degradation time of 5000 ms was used for NPL. The circularity of the features formed under each condition was utilized as a measure of feature shape replication fidelity. Small features displayed a circularity factor close to one (essentially round), while larger features had a circularity of about 0.4 for all conditions (Figure 4a). While no difference was conveyed in the circularity of features formed under the different conditions, the star-shape geometric resolution of features formed

in the ambient PPL 40× and NPL conditions visually appear to be superior to the ambient and purged PPL 20× conditions (Figure 4b–e). Improved resolution in the NPL condition was expected since patterning by degradation does not depend upon the formation or consumption of initiator radicals, only the optical intensity field. Improved resolution was observed when using a higher magnification to form features under PPL conditions, presumably due to higher UV intensity and therefore more rapid and complete photopolymerization. This demonstrates that NPL can not only produce accurately sized features, but is capable of fabricating spatially complex features with excellent fidelity. These results reinforce the interplay between projected UV area and exposure time in accurately determining size as well as the fidelity of polymerized features. Overall, NPL allows for the formation of complex features with good fidelity, which was not achievable with PPL.

In Situ Chemical Microenvironment Modification by Post Degradation.

Utilizing photodegradable PEG as an in situ fabrication material not only allows the microfabrication of features with excellent fidelity, but also introduces the ability to add functionality to biomicrofluidic systems with spatiotemporal precision through the release of encapsulated or conjugated biomolecules from formed hydrogel networks. Here, we introduced the ability to dynamically reconfigure sealed microfluidic systems by eroding photodegradable hydrogel posts. Photodegradable hydrogel posts containing acrylated Rhodamine B were successfully fabricated as biomolecular reservoirs and subsequently degraded by exposure to 30 s of long wavelength UV light. The release and diffusion of Rhodamine into the surrounding medium created a temporary circular gradient that was monitored via fluorescence microscopy. The relative fluorescent intensity was determined for the original post area, measured from the origin (0) to the radius (r) of the post, and the area surrounding the original post, measured from r to r plus 100 μm (R), for all time points (Figure 5a). As the relative fluorescent intensity of the original post structure decreased following degradation, the fluorescent intensity outside the original post area increased (Figure 5). The relative fluorescent intensity of both areas collapse to a similar value after 10 min, indicating that a homogeneous Rhodamine concentration was achieved following its diffusion out of the original post area and into the surrounding media. As Rhodamine was covalently conjugated to the network the diffusion profile observed here can be solely contributed to the cleavage of the nitrobenzyl-linker within the hydrogel.

Photolabile PEG posts that contain encapsulated or conjugated biomolecules for on-demand release can be utilized to alter the biochemical microenvironment toward applications for tissue engineering, drug delivery, and biosensing. The ability to easily incorporate, retain, and release a wide range of biomolecules (e.g., small or large proteins, peptides, etc.) makes this a versatile and robust technology. Thus, the biochemical makeup of a system be controlled using photodegradable hydrogel posts, which produce biocompatible degradation products and functional, active macromolecules.^{66,67} Additionally, multifunctional arrays could be fabricated to release multiple chemicals or biomolecules either simultaneously or sequentially for complex spatiotemporal control over a microfluidic system's state. Further, to create a sustained gradient within the device the applied light dose could be altered (e.g., decreasing the light intensity or using a stepped light dosage application) leading to posts

that are either partially or fully degraded to provide user-defined biomolecule release profiles.

Posts with Layered Functionality.

Photodegradable hydrogels also enable the fabrication of three-dimensionally patterned structures without the need to precisely align photomasks over repeated photopolymerization steps. This unique capability is demonstrated with the photolithographic patterning of multilayer hydrogel posts by NPL (Figure 6). Individual hydrogel layers, each containing distinct functional compositions, were formed by successively polymerizing hydrogel films within PDMS microfluidic molds. Subsequently, NPL was used to erode the layered films into structured pillars with uniform width and shape throughout their depth. Here, alignment of the hydrogel films is facile and features may be formed anywhere in the film based upon the mask positioning in a single NPL UV exposure step. This is in stark contrast with PPL where each layer requires repeated, exact mask alignment to accurately form small multilayered features.

The incorporation of distinct components into each compartment was demonstrated by the incorporation of Rhodamine B into the top layer and fluorescently labeled bovine serum albumin (BSA) into the bottom layer. Without the need to precisely align the photomask for photopatterning, the ease of fabrication, as well as the fidelity and reproducibility of multilayered features, is increased relative to PPL. The facile fabrication of multilayered structures using NPL provides a unique and accessible method for engineering complex and dynamically reconfigurable microenvironments. This fabrication approach may prove useful in a wide range of applications in basic and applied biology including sequential release of biochemical cues to cells to study cellular processes or in the development of multifunctional bioassays.

CONCLUSION

We demonstrated and quantified the in situ formation of PEG hydrogel microstructures within microfluidic channels using both positive and negative projection lithography with photopolymerizable and photodegradable PEGDA, respectively. PEGDA hydrogel microstructures were formed by photopolymerization with PPL within PDMS devices; however, the process was observed to impose severe limitations upon the resolution, size, and complexity of the features. The limits imposed upon PEG microstructure fabrication within PDMS devices using PPL were quantitatively demonstrated to be primarily the result of radical diffusion and oxygen inhibition during the polymerization reaction. To avoid the limitations of PPL in forming microstructures, we introduced NPL, the microfabrication of hydrogel structures using photodegradable PEG. NPL was utilized to form PEG microstructures with superior size control, higher resolution, and pattern replication fidelity in comparison to those formed by PPL. Beyond scale and resolution, NPL was demonstrated to provide several key advantages over PPL, including the ability to noninvasively degrade microstructures for the formation of dynamic chemical gradients and the generation of microstructures with complex patterns and multiple functionalities.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Figure 1.

Schematics of positive and negative projection lithography. (a) To form hydrogel features using PPL, a projection mask that allowed for light to pass through the mask in the polymerization areas of interest was used. After light irradiation to polymerize the hydrogel forming solution, raised hydrogel features on glass were formed. (b) Hydrogel features were formed by NPL using a shadow mask, which allowed for light to pass around the features of interest. Because these features were formed using a subtractive method, a photodegradable hydrogel post larger than the final feature size was formed by irradiating with visible light through a partially closed microscope iris (represented by orange hexagon). Next, the post was degraded by exposure to long wavelength UV light passed through the shadow mask to leave behind raised hydrogel posts on glass. Representative images of features formed using PPL (c) and NPL (d) are shown. Scale bars are 25 μm .

Figure 2.

Effects of long wavelength UV exposure time on feature area. Hydrogel features were formed using either a mask projection area of 775 (a) or 22 μm^2 (b) and the area of the resulting hydrogel was determined. The mask projection area is represented by a dashed line and illustrated in the top left corner of the graph. In all cases, we observed that the size of features formed using PPL, independent of oxygen presence, was larger than the mask projected area. Conversely, the area of the features formed using NPL tended to be slightly smaller than the mask shadowed area and generally closer to the mask area. Representative features formed using the 20 $\times$ objective under ambient (c) or nitrogen purged (d) conditions using PPL and features formed using NPL (e) are shown. Under each condition, the left image shows the features formed with an expected area of 775 μm^2 and the right image (smallest features) shows the features formed with an expected area of 22 μm^2 . Scale bars are 25 μm .

Figure 3.

Comparison of expected and actual hydrogel feature area. (a) The percent difference in the length of hydrogel features formed compared to the mask projected or mask shadowed length for PPL and NPL, respectively. It was observed that features formed using PPL were consistently larger than the projected feature where those formed using NPL were consistently smaller. (b) Double bond conversion fraction results from COMSOL model. Each line indicates a new time step starting at 0 ms and going to 1000 ms in 100 ms increments. The dotted line represents the mask projected post radius (R), which is defined as -12.5 to 0 in the model. The x -axis shows R subtracted from arc length (r). Any polymerization outside the mask projected radius is due to diffusion of radicals into the surrounding solution.

Figure 4.

Formation of complex geometric features. (a) The effect of feature size and hydrogel formation conditions on the circularity of star microstructures was investigated. The size of features investigated was dependent on the microscope objective and PL technique. The areas investigated were 5.4 μm^2 , 335 μm^2 , and 775 μm^2 for the 20 $\times$ PPL conditions, 0.8 μm^2 (*), 50 μm^2 (**), and 150 μm^2 (***) for the 40 $\times$ PPL condition, and 22 μm^2 (*), 335 μm^2 , and 775 μm^2 for the NPL condition. Features were polymerized in PPL conditions with an exposure time of 100 ms in a 50 μm deep channel. A degradation time of 5000 ms in a 20 μm deep channel was used for the NPL condition. Overall, no difference in the circularity of the features was determined among the different conditions tested, but there were observed differences in the feature resolution and shape. Representative images of the features formed using PPL ambient 20 $\times$ (b), PPL purged 20 $\times$ (c), PPL ambient 40 $\times$ (d), and NPL (e) conditions are shown. Scale bars are 20 μm .

Figure 5.

In situ modification of the chemical microenvironment. (a) The chemical microenvironment within the microfluidic device was modified by exposing a photodegradable PEG post containing Rhodamine to 30 s of long wavelength UV light. Normalized relative fluorescent intensity of the area inside the original post area and outside the original post area was used to determine the diffusion of Rhodamine into the surrounding media. The profile created was monitored over 10 min. As time increased, we observed a decrease in the fluorescence in the original post area and an increase in the area outside the initial post area indicating the diffusion of Rhodamine into the surrounding environment. Images are shown for 0 s (b, before UV exposure), 30 s (c, directly after UV exposure), and 600 s (d, after 10 min of diffusion time). Scale bars are 100 μm .

Figure 6.

Formation of multilayered hydrogel posts. (a) Schematic of the setup used to create multilayered hydrogel posts using NPL. Two large hydrogel posts were polymerized on top of one another and subsequently exposed to long wavelength UV to form small layered hydrogel features. (b) The 3D reconstruction of confocal z-stacks shows small layered hydrogel features where the first layer contains Rhodamine and the second layer contains BSA-488. The inset image shows an x - y slice of a post structure. Scale bar is 10 μm .