Chapter 24

Multilayer Microfluidic Poly(Ethylene Glycol) Diacrylate Hydrogels

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Abstract

Development of robust, in vivo like tissues in vitro holds the potential to create regenerative medicine-based therapeutics, provide more physiologically significant preclinical models and supply a pharmacological and toxicological screening platform that reflects in vivo systems in both complexity and function. This protocol describes a simple, robust, multilayer replica molding technique in which poly(dimethylsiloxane) (PDMS) and poly(ethylene glycol) diacrylate (PEGDA) are serially replica molded to develop microfluidic PEGDA hydrogel networks embedded within independently fabricated PDMS housings, using a combination of soft and photo-lithography. This work has direct applications toward the development of robust, complex, cell-laden hydrogels for in vitro diagnostics and regenerative medicine applications.

Key words: Hydrogel, Poly (ethylene glycol), Microfabrication, Microfluidics, Photolithography, Soft lithography

1. Introduction

Microscale systems are advantageous to the life science field due to their ability to reduce reagent volume, increase system throughput, and allow investigation of microscale cellular phenomena (1–3). In particular microfluidic systems provide picoscale fluid volume transfer, robust control of molecular gradients, and miniaturization of complex multicomponent systems (4, 5). However, a majority of microfabricated systems use 2D substrates (6, 7) or are based on silicone elastomers that are not favorable for cell culture and tissue engineering applications (8, 9). Integration of microfabrication technologies with hydrogel materials that are biocompatible and biomimetic enables more accurate modeling of living systems in vitro (10–12). Applications of microfabricated hydrogels range

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from regenerative medicine therapeutics (13) to more clinically relevant tissue models for drug screening applications (14, 15). Previously we have shown the ability to fabricate multilayer polydimethylsiloxane/poly(ethylene glycol) diacrylate (PDMS/PEGDA) hydrogels (16). Herein we describe methods to fabricate PEGDA hydrogels within PDMS housings using a combination of soft and photo-lithography.

The methods employed to build microfluidic or other microfabricated hydrogels vary widely depending on the application. The choice of hydrogel material and the mechanism that drives cross linking from a pre-polymer solution to a gel will alter procedures significantly (12, 16, 17). We have fabricated poly(ethylene glycol) (PEG) microfluidic networks with polymer concentrations ranging from 2.5 to 50% (w/v) at molecular weights ranging from 575 to 20,000 Da. The methods reported below will focus on the photopolymerizable derivative of PEG, poly(ethylene glycol) diacrylate (PEGDA), which is crosslinked using a photoinitiator that generates a free radical and initiates crosslinking upon exposure to ultraviolet (UV) light. Much care must be taken when choosing the most appropriate polymer, photoinitiator, and light sources for a given application. For a more detailed description of photoinitiators and photopolymerizable PEG derivatives please refer to West et al. (18).

Two hydrogel microfabrication approaches, using both soft and photo-lithographic techniques, will be described in detail. The first process describes the steps to independently fabricate a microfluidic hydrogel network within a robust polydimethylsiloxane (PDMS) perfusion housing using soft lithographic techniques. The second process describes the steps to photolithographically fabricate hydrogel microstructures within a PDMS housing followed by a soft lithographic step to independently fabricate microfluidic networks. This type of multimodal fabrication scheme will allow for the production of increasingly complex materials and highly biomimetic systems, which are important for the development of more relevant preclinical in vitro tissue models and for the production of complex, regenerative medicine-based therapeutics.

Depending on the chosen application and the required microfabrication tolerances, the use of a clean room facility may be required for the production of photoresist masters. Generally, we have found that use of clean room facilities produces robust, high fidelity, and long-lasting photoresist masters. However, much recent effort has been placed on adapting microfabrication technologies for out of clean room bench top applications (19–21). Herein, all photoresist masters were fabricated within a clean room but replica molded PDMS housings, PEGDA microchannels, and photolithographically fabricated PEGDA microstructures were produced on the bench top.

2. Materials

2.1. Photoresist Master Fabrication

1. Glass slides or other silicon-based substrates (see Note 1 on substrate resist adhesion effects).
2. Three glass slide staining dishes with one removable glass slide rack.
3. Piranha solution: concentrated sulfuric acid (18 M) and 30% hydrogen peroxide in a 7:3 v/v ratio (sulfuric acid:hydrogen peroxide) (see Note 2 on piranha handling and safety precautions).
4. SU-8 2100, SU-8 2050, and SU-8 developer (Microchem) protected from light and stored at room temperature.
5. Acetone (ACS grade), 2-propanol (ACS grade), and ultrapure water stored in standard 500 mL low density polyethylene wash bottles.
6. Temperature programmable 200°C hot plate (VWR).
7. Spin coater (PWM32-PS-R790, Headway Research).
8. Transparency photomask (25,000 dpi, CAD/ART Services).
9. Collimated mask aligner light source (MJB4 Mask Aligner, SUSS Microtec).
10. Filtered and dry nitrogen.
11. Sigmacote (Sigma Aldrich) protected from light and stored at 4°C in a flammables safe refrigerator.

2.2. Polydimethylsiloxane (PDMS) Housing Fabrication

1. Polydimethylsiloxane (PDMS, Sylgard184 kit, ESI, Inc.).
2. Round Petri dish (100×25 mm, polystyrene).
3. Disposable spatula or disposable serological pipette.
4. Vacuum pump and desiccator or oven with vacuum chamber.
5. 1 mm biopsy punch (see Note 3 on compression fitting).
6. Razor blade or scalpel.
7. Acetophenone (2,2-dimethoxy-2-phenyl acetophenone, Sigma Aldrich).
8. NVP (*n*-vinylpyrrolidone, Sigma Aldrich).
9. Ethanol (100%, ACS grade), and ultrapure water stored in standard 500-mL low density polyethylene wash bottles.
10. Filtered air.

2.3. Soft Lithographically Fabricated Poly(Ethylene Glycol) Diacrylate Hydrogel

1. Long wavelength UV lamp with spot bulb (365 nm, 10 mW/cm^2, Blak-Ray).
2. Poly(ethylene glycol) diacrylate (PEGDA) (see Note 4 on hydrogel content).
3. Phosphate-buffered saline (PBS, pH 7.4).

4. Irgacure 2959 photoinitiator (Ciba).
5. 1 mL syringe w/luer lock.
6. Polyethylene tubing (OD 1 mm ID 0.58 mm, see Note 3 on compression fitting).
7. Ethanol (100%, ACS grade).
8. Coverglass or glass slide.
9. Syringe pump with infusion and withdrawal mode (Harvard Apparatus Holliston, MA).

2.4. PEGDA Hydrogels Fabricated Using Photolithography and Soft Lithography

1. Long wavelength UV lamp with spot bulb (365 nm, 10 mW/cm^2, Blak-Ray).
2. Poly(ethylene glycol) diacrylate (PEGDA) (see Note 4 on hydrogel content).
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6. Ethanol (100%, ACS grade).
7. Coverglass or glass slide.
8. Syringe pump with withdrawal mode (Harvard Apparatus).

3. Methods

The following methods describe multilayer soft lithographic and photolithographic fabrication methods to create PEGDA microfluidic hydrogels contained within a PDMS housing see Note 5 for design considerations.

3.1. Photoresist Master Fabrication

1. Load pre-cleaned glass slides into the staining rack and place them in a glass staining tray. Carefully add approximately 250 mL (or enough volume to cover the slides) of piranha solution into the staining tray. Allow the piranha solution to cleanse and etch the glass for 15 min with periodic, gentle agitation of the slide rack (see Note 2 on piranha handling and safety precautions).
2. Remove the rack of glass slides from the piranha solution and rinse in a staining tray of ultrapure water for 1 min while constantly and gently agitating. Next move the rack to a second tray of ultrapure water and allow the cleaned slides to soak for 5 min during which time the water will hyrdoxylate the glass surfaces.
3. Individually remove each slide from the water using tweezers, thoroughly dry with nitrogen and then place on a hot plate at 200°C for at least 10 min to dehydrate the slide surface.

4. After heating allow the slides to equilibrate to room temperature for at least 2 min.
5. Center a slide on the spin coater stage taking care to only handle it by the edges. Activate the spin coater vacuum to hold the slide in place. The dominant factors governing structure height are the choice of the resist and the speed and length of time chosen for the spin coating step. Please see Microchem's website (http://www.microchem.com/Prod-SU8_KMPR.htm) for parameter details (see Note 6 on proper protective equipment and spin coater operation).
6. To create the PDMS housing master, slowly pour the SU-8 2100 onto the substrate, making sure to coat the entire surface without trapping any bubbles. This is best achieved by a single, thick pour with the resist bottle spout almost touching the slide. Set the spin coater to a final spin speed of 1,000 rpm with a ramp speed of 300 rpm/s and a 40 s spin time (Fig. 1, Step 1).
7. Carefully remove the PDMS housing SU-8 2100 master from the spin coater stage and place it onto a hot plate that is at room temperature. Turn the hot plate to 65°C and bake for 30 min once the hot plate reaches temperature. Turn the temperature on the hot plate up to 95°C and bake for 120 min once the temperature reaches 95°C (see Note 7 on hot plates and the effects of thermal fluctuations).
8. To create the PEGDA microchannel master, repeat Subheading 3.1, Steps 5–7 with the following modifications. In Subheading 3.1, Step 6: replace SU-8 2100 with SU-8 2050. For the bake times in Subheading 3.1, Step 7: use 5 min at 65°C and 30 min at 95°C, taking care to gradually heat the substrate and allow the temperature to equilibrate before starting the timer.
9. Remove the substrate from the hot plate and cool to room temperature for 2 min. Place the coated substrate onto the mask aligner chuck or sliding stage. Mount the transparency mask with the ink side of the transparency in direct contact with the SU-8 surface on the slide (Fig. 1, Step 2) (see Note 8 on exposure light source).
10. The UV exposure time is dependent upon the type and thickness of the photoresist, the substrate used, and the energy output of the mask aligner lamp. Please see manufacturer recommendations for these materials and alter parameters as needed for optimal resist performance. A simple formula can be used to calculate the exposure time for a given exposure energy and lamp power.

$$\text{Exposure Time (s)} = \frac{\text{Exposure Energy}\left(\frac{\text{mJ}}{\text{cm}^2}\right)}{\text{Lamp Power Output}\left(\frac{\text{mW}}{\text{cm}^2}\right)}$$

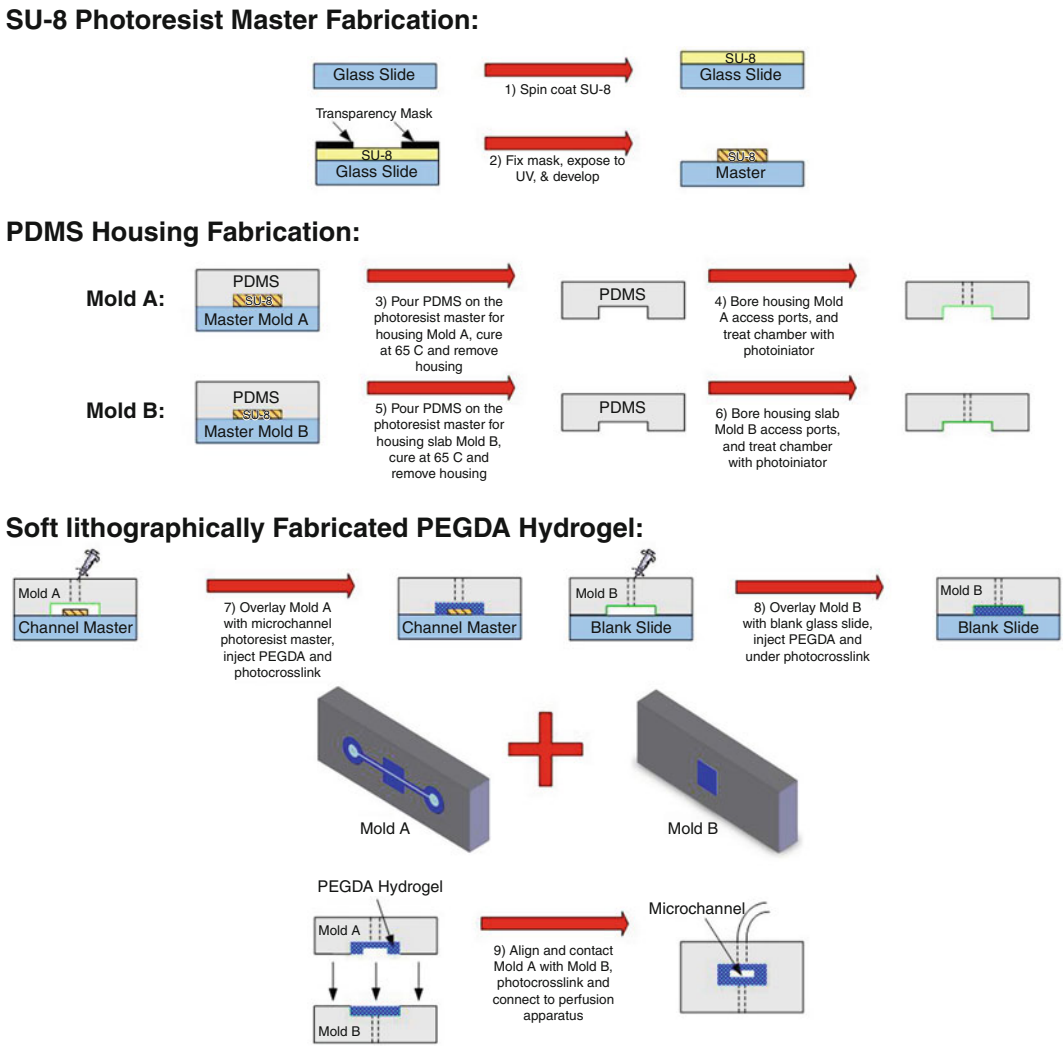

Fig. 1. Fabrication schematic of PDMS/PEGDA microfluidic networks. Serial replica molding of PDMS and PEGDA to photoresist masters produces a perfusable PEGDA microfluidic channel embedded within an exterior PDMS housing. The PDMS/PEGDA construct, Mold A, can be sealed to coverglass for imaging after Step 5 or crosslinked to a PEGDA blank slab, Mold B, to produce a 3D microchannel environment **(**Step 9**).**

11. After the resist is exposed, place the slide onto a hot plate at room temperature. For the PDMS housing SU-8 2100 master, set the hot plate to 65°C and bake for 5 min once the plate reaches this temperature. Next turn the hot plate up and bake for 20 min at 95°C. For the PEGDA microchannel SU-8 2050 master, the bake times are reduced to 65°C for 5 min and 95°C for 10 min. As before, take care to gradually heat the substrate and allow the temperature to equilibrate before beginning the bake time in each step.

12. Turn off the hot plate and allow the resist to cool to room temperature. Place the substrate into a "puddle" of SU-8 developer in a glass Petri dish and gently swirl or rock the dish intermittently while developing. Use a developer volume that is sufficient to fully cover the substrate.
13. Development times will vary depending on the thickness of the resist and the area of nonexposed photoresist that needs to be dissolved. For the PDMS housing SU-8 2100 master, the development time can be up to 60 min, with a recommended replacement of the developer solution after 30 min. The SU-8 2050 master is usually completely developed in 30 min and does not require a change of developer solution.
14. Remove the resist from the developer puddle and rinse with 2-propanol. White residue or precipitate that becomes visible upon washing is indicative of undeveloped resist and requires additional developing time. It may be necessary to repeat the development and 2-propanol rinse steps multiple times until no precipitate is formed (see Note 9 on developing and fine structure development).
15. Once the resist is fully developed, rinse it with ultrapure water and dry with filtered air or nitrogen.
16. This entire section (Steps 1–15) can be repeated with various combinations of PDMS housings and microchannel masters to create a wide variety of system geometries and configurations.

3.2. PDMS Housing Fabrication

1. Rinse a Petri dish and SU-8 2100 PDMS housing photoresist master with pure ethanol and dry with filtered air.
2. Mix PDMS at a 15:1 ratio of elastomer:curing agent using a disposable plastic spatula for at least 1 min or until trapped bubbles are homogenously dispersed within the mixture. Higher (20:1) and lower (10:1) ratios of elastomer:curing agent can be used to produce softer or stiffer housings, respectively. For more detailed information on silicone properties and reagents for silicone functionalization, see the commercial website http://www.gelest.com/gelest/forms/Home/home.aspx.
3. Place the PDMS housing master into the Petri dish and pour the PDMS mixture over the mold, making sure to recover all of the polymer (Fig. 1, Step 3) (See Note 10).
4. Next, degas the mixture in a vacuum oven (−30 psi) at room temperature for 1 h or until all bubbles are removed.
5. After degassing transfer the mold to a 60°C oven and cure for 3 h. A more rapid curing (1 h) can be accomplished at 100°C but this temperature is not suitable for the polystyrene Petri dishes.

6. Separate the PDMS housing from the photoresist master and the Petri dish using a razor blade. Bore access ports in the PDMS housing at the desired location using a 1-mm biopsy punch (Fig. 1, Step 4).
7. Finally, rinse the PDMS housing with ethanol and conformally seal it to a pre-cleaned glass slide until further use (see Note 11 on reversible conformal seals).
8. Repeat Steps 2–7 for each PDMS housing component that is required in the final design. Note that for a 3D hydrogel environment, as shown in Fig. 1, Mold A forms the template for the housing with the channel in relief (Fig. 1, Steps 3 and 4) while Mold B forms the blank slab for the device bottom (Fig. 1, Steps 5 and 6).

3.3. Soft Lithographically Fabricated Poly(Ethylene Glycol) Diacrylate (PEGDA) Hydrogel

1. Dissolve PEGDA in PBS at the desired concentrations and add the Irgacure 2959 photoinitiator (100 mg/mL in ethanol) to the hydrogel pre-polymer solution at 30 μL/mL (see Note 4 for choice of hydrogel composition).
2. Insert polyethylene tubing with an attached syringe into one of the PDMS housing access ports in Mold A, and inject the acetophenone photoinitiator (300 mg/mL in NVP) until the entire housing is filled. Allow the photoinitiator solution to coat the housing for 5 min.
3. To remove excess photoinitiator, peel the PDMS housing from the glass slide and rinse with ethanol and then ultrapure water before drying the device with filtered air.
4. Treat the SU-8 2050 photoresist master to be used as the PEGDA microchannel mold with Sigmacote for 1 min. Rinse with ethanol, and then dry with filtered air (see Note 12 on engineering interfaces).
5. Overlay and align the acetophenone coated PDMS housing with the SU-8 2050 microchannel photoresist master (Fig. 1, Step 7).
6. Inject the PEGDA pre-polymer solution with without cells (see Note 13) into the housing around the channel mold and expose the device to a long wavelength UV lamp (365 nm, 10 mW/cm^2) for 2.5 min (Fig. 1, Step 7), in order to form the 3D microfluidic network.
7. Next, conformally seal the acetophenone coated PDMS housing for the blank slab (Mold B) against a Sigmacoted glass slide. Inject the PEGDA pre-polymer solution with or without cells into the housing and crosslink using a short 1 min UV exposure time. This will form a hydrogel slab that will serve as the base of the hydrogel device (Fig. 1, Step 8).

8. Finally, overlay Mold A containing the PEGDA microchannel hydrogel with the under-crosslinked PEGDA slab (Mold B) and expose to UV for 2.5 min to graft the two components together (Fig. 1, Step 9). Alternatively, if a 3D hydrogel environment around the channels is not required, the PDMS housing containing PEGDA microchannels in relief can be conformally sealed to cover glass after Subheading 3.3, Step 6 to allow ease of high resolution imaging.
9. To activate the microfluidic device, first connect polyethylene (PE) perfusion tubes to the PDMS inlet/outlet access ports, perfusion reservoir, and syringe pump. Apply quick-dry 5 min epoxy at the PE tube/PDMS interface to help anchor the tubes and prevent pulling on the compression fit. Initiate perfusion at 600 μL/h using a syringe pump (Harvard Apparatus) in withdrawal mode. See the Chips and Tips section of the Lab on a Chip journal website for helpful and pragmatic tips on a wide variety of microfabrication techniques including alternative ways to interface tubing with chips (http://www.rsc.org/Publishing/Journals/lc/Chips_and_Tips/interfacing.asp).

3.4. PEGDA Hydrogels Fabricated Using Photolithography and Soft Lithography

Soft lithographic hydrogel fabrication methods described in Subheadings 3.2 and 3.3 can be combined with photolithographic fabrication methods to build multilayer and multicomponent PEGDA hydrogel structures within a PDMS housing.

1. Fabricate PDMS housing as described in Subheading 3.2 and treat with photoinitiator as described in Subheading 3.3, Steps 2 and 3.
2. Conformally seal the coated PDMS housing to a Sigmacoted glass slide. Insert a PE tube connected to a syringe into one of the punched access ports and inject the PEGDA solution to be used for the first set of structures into the housing (Fig. 2, Step 3, PEGDA Solution 1).
3. Align the transparency photomask with patterns for PEGDA Structure 1 with the PDMS housing and expose to UV light for 2.5 min (see Note 14 on alignment, light source, and alternative techniques).
4. After exposure, peel the PDMS housing with attached PEGDA structures from the Sigmacoted glass slide and gently rinse with ultrapure water (use buffer if cells are present). Gently dry the device with filtered air.
5. Figure 2, Steps 2–4 can be serially repeated with PEGDA hydrogel solutions of different content and photomasks with different structure geometries to produce multiple, independently fabricated PEGDA structures.

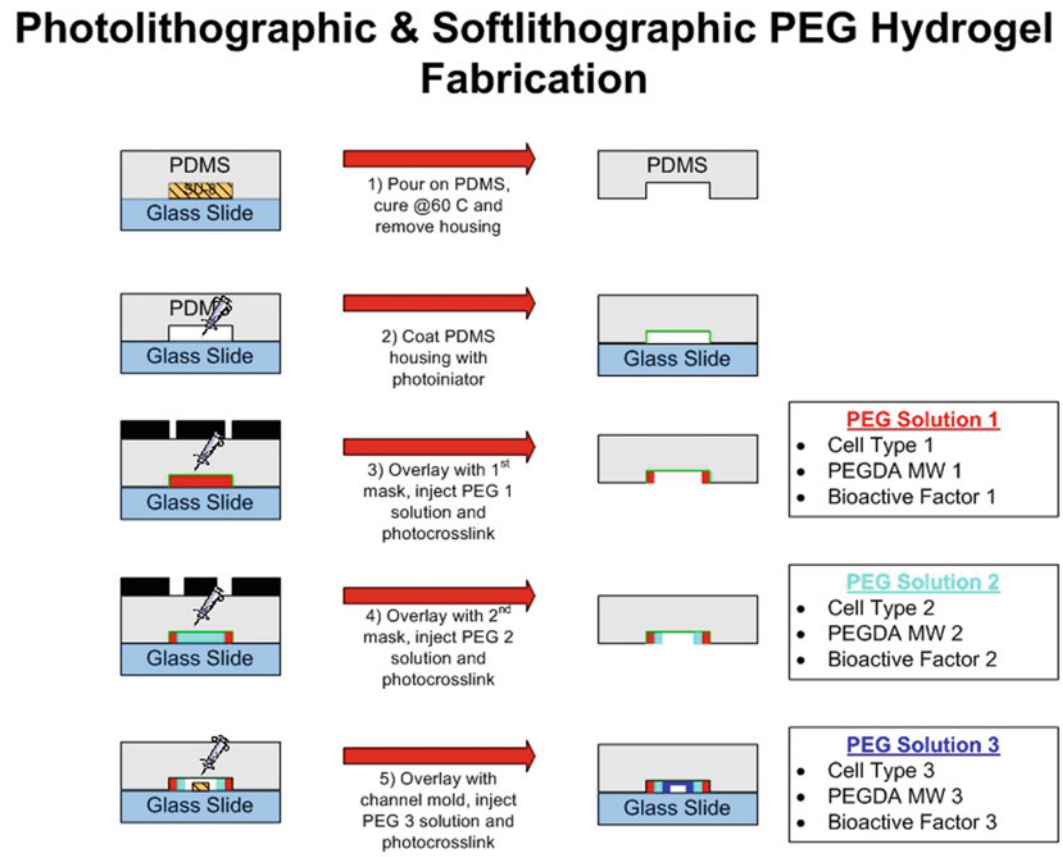

Fig. 2. Photolithographic and soft lithographic fabrication schematic of PDMS/PEGDA microfluidic networks. Serial mask-directed photopolymerization of PEGDA pre-polymers produces hydrogel structures with various physical and bioactive content (steps 3 and 4). Soft lithographic replica molding of PEGDA to a photoresist master produces a perfusable PEGDA microfluidic channel (Step 5).

6. Overlay the PDMS housing with attached PEGDA hydrogel structures with a Sigmacoted SU-8 2050 photoresist microchannel master (Fig. 2, Step 5).
7. Insert PE tubing with attached syringe into one of the punched PDMS housing access ports and inject the final PEGDA solution into the housing. Expose the device to a UV light source for 2.5 min.
8. Remove the PDMS housing with multiple PEGDA structures and PEGDA microchannels from the photoresist master and conformally seal to cover glass. Connect PE tubes and initiate perfusion. Example images of multilayer PEGDA Hydrogels are shown in Fig. 3.

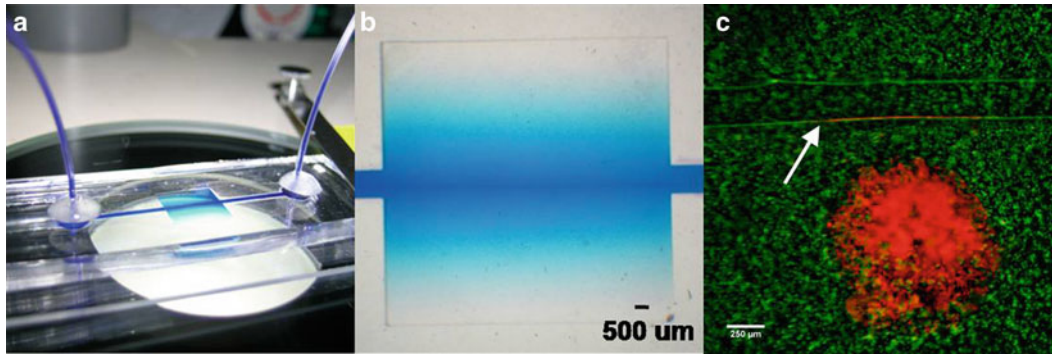

Fig. 3. (**a**) Multimodal fabricated PEGDA hydrogels. Isometric view of PDMS/PEGDA microchannel device perfused with *toluidine blue*. (**b**) Diffusion of *toluidine blue* into PEGDA diffusion chamber (10 × 10 mm) from a single 50 µm channel. Reproduced by permission from Elsevier. Cuchiara et al. (16). (**c**) Multimodal fabrication of murine hippocampal progenitor cell pillars (MHP 36, *red*) and human umbilical vein endothelial cells (HUVECs, *green*) replica molded to form a microfluidic channel (*arrow*).

4. Notes

1. Adhesion strength of SU-8 to various substrates can be found at Microchem's website (http://184.168.52.107/pdf/SU-8-table-of-properties.pdf). These effects will become more pronounced as resist structure aspect ratio (height/width) increases. The author has chosen to use glass because it is readily available and its performance is adequate for the given application.
2. Piranha is a very dangerous solution that is highly acidic and a strong oxidizer. Reaction of sulfuric acid and hydrogen peroxide is exothermic and the resulting solution can be explosive. Extreme caution must be employed to protect both the user and the user's workspace. Please thoroughly research piranha handling and disposal procedures before carrying out the protocols in this chapter.
3. In order to prevent leaking at the tube chip interface, a compression fit between the perfusion tubing and bored access port must be established. A compression fit requires that the diameter of the biopsy punch or access port be slightly less than or equal to the perfusion tubing outer diameter. The authors have found the a 1 mm biopsy punch combined with 1 mm OD PE tubing will hold a tight seal but can also be easily inserted.
4. Perhaps the most understated aspect of the protocols is the choice of PEGDA hydrogel system that satisfies the functional needs of the desired application. A rapidly growing body of PEGDA hydrogel research across disciplines has reported a wide variety of PEGDA hydrogel compositions that are suitable

for the photo and soft lithographic fabrication techniques described in this chapter. The reader is encouraged to start with a hydrogel composition that satisfies the desired application and work to design and optimize fabrication protocols around this target hydrogel composition. The reader is encouraged to reference book chapters by Miller et al. (22) and Bryant et al. (23) for more detailed information on photopolymerizable hydrogels.

5. Design of multilayer PDMS-PEGDA systems is dependent upon the differential geometry of the SU-8 photoresist master structures used to mold the PDMS housing and the SU-8 photoresist master structure used to mold the PEGDA channel (Fig. 1). Each user will have to define their structure geometry for a given application. The *x*–*y* plane geometry used will vary greatly from study to study and is easily modified by changes to the photomasks. The *z*-plane or structure height is determined by photoresist processing parameters and is not as readily tuned. The author has found that structure height of 350 μm for the PDMS housing mold and 100 μm for the PEGDA microchannel mold provides reliable perfusion and aspect ratios well within the manufacture recommended specifications. All process parameters mentioned herein are optimized from manufacturer recommended protocols. The reader is encouraged to reference Microchem's website for detailed information on adjustments to processing parameters and other helpful tips: (http://www.microchem.com/Prod-SU8_KMPR.htm).
6. Always operate the spin coater with a cover over the spinning chamber and use eye protection.
7. One of the most influential parameters on photoresist structure height, quality, and adhesion to the substrate is proper baking practices. Minimization of any rapid thermal fluctuations between baking steps and slowly ramped temperature baking protocols will go a long way to improving photoresist performance. In addition, the author has found that the temperature profile across a hot plate is not constant with the center of the hot plate likely being at the target temperature, while regions closest to the hot plate edges may show a decrease in temperature between 10 and 30% of the target temperature. Take care to make sure your substrate is experiencing the desired baking temperature. The author has found that the purchase of a standard infrared thermometer is a useful tool in validating bake temperatures and hot plate thermal profiles.
8. Various light sources can be used to expose photoresist. It is important that the spectral output of the light source corresponds to the photoresist's absorption spectrum. It is also important that the light source used is collimated. Mask aligner instrumentation with micromanipulators and imaging capability aid with multilayer alignment but are usually not necessary for single exposures.

9. The use of a standard light microscope periodically during photoresist development step may be helpful in determining when the photoresist structures are completely developed. The presence of a white film at the edge of a structure is indicative of undeveloped resist. For structures in close proximity, especially narrow "troughs" or "holes," more aggressive agitation, such as sonication, may help to remove undeveloped resist.
10. PDMS is very difficult to clean from glassware and other lab tools. It highly recommended that all materials used to prepare and cure PDMS be disposable.
11. For PDMS-PEGDA hydrogel microdevices, the PDMS-substrate seal must be reversible and methods for sealing the hydrogel laden PDMS chip must be cell compatible. The author has found that the only way to produce a PDMS-PEGDA microdevice that forms a reversible seal and does not leak is through a conformal seal. Conformal seal performance requires that the PDMS housing and substrate surfaces are very clean and free of particles. The best conformal seals form passively and do not require application of manual pressure. Conformal seals have been shown to hold a positive pressure of 5 psi (24) but the author has found that the most robust method for maintaining a conformal seal is the continuation of negative pressure via a syringe pump in withdrawal mode. This can be the same negative pressure used to drive flow through your device (16) or it can be a separate design that is only used to seal the interface and keep the chip from leaking (25).
12. Another understated aspect in this process is the need to selectively engineer adhesive and nonadhesive interfaces. In order to facilitate PEGDA polymerization at the PEGDA/PDMS interface and to promote PEGDA adhesion to the PDMS, the author recommends treating the PDMS housing with the photoinitiator acetophenone in the solvent NVP. It is thought that the NVP dissolves the PDMS polymer structure therefore facilitating PEG-PDMS chain–chain entanglement. Furthermore, the presence of locally high concentrations of acetophenone and the free radical acceptor NVP will counteract the free radical quenching effect of oxygen dissolved in the PDMS matrix. Other groups have recommended functionalizing the PDMS surface with acrylates (26) or coating with oxygen impermeable parylene c (27) to promote hydrogel polymerization at the PDMS interface. For a more complete review on PDMS surface functionalization please refer to Hu et al. (26). Conversely it is necessary to engineer a nonadhesive interface in order to avoid hydrogel tearing during the molding of PEGDA microstructures from SU-8 photoresist masters or blank glass slide substrates. The author has found that the commercially available Sigmacote provides a robust, easy-to-use, nonadhesive coating that improves the reliability of the PEGDA molding process.

13. All system components that are to contact cells or cell solutions must be sterilized prior to use. All flow loop components, including the PDMS chip, PE tubing for perfusion, valves for flow control, and reservoir bottles that are left in place during the duration of the cell culture are sterilized by either ethylene oxide exposure or autoclaving. Components that are only temporarily used during the fabrication process are sterilized under UV light in a laminar flow biosafety cabinet for at least 12 h. These components include PE tubing for solution injection, Sigmacoted glass slides, 200 proof ethanol and ultrapure water wash bottles, and UV lamp for polymerization. The PEGDA pre-polymer solution is sterile-filtered using a 0.22 μm PES syringe filter to remove debris and containments.
14. As with all multilayer lithography, multistructure fidelity is often limited by the ability to align the photomask with existing structures. The author has found that resolving >1 mm alignment tolerances is possible "by eye" with practice. However, when aligning multiple structures with micron-level precision, it is necessary to use a micromanipulator or a common microscope stage controller coupled with a microscope. The author has found that the use of a mask projection photolithography technique (28) allows for micron-level manipulation and alignment of the substrate with a single wavelength of light followed by exposure of the pre-polymer solution using the same light path with a different wavelength of light. This technique allows for precise alignment and is compatible with newer three-dimensional laser scanning lithography fabrication techniques (29).

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