

Subtractive Methods for Forming Microfluidic Gels of Extracellular Matrix Proteins

Gavrielle M. Price and Joe Tien

Department of Biomedical Engineering, Boston University, 44 Cummington Street, Boston, MA 02215

Abstract

Microfluidic networks in extracellular matrix hydrogels hold great promise in tissue engineering. We have established two methods for forming microscale channels within collagen and fibrin gels. Both methods rely on molding gels around removable elements: stainless steel needles for creating single channels and gelatin meshes for forming networks of interconnected channels. These methods can form single channels $50\mu\text{m}$ or more in diameter or networks $6\mu\text{m}$ or more in width, at a rate of 5 to 10 samples per day. Potential applications include using microfluidic gels for engineering microvascular networks, perfusing cultured cells within native scaffolds, and controlling interstitial flows in cell culture models.

Key terms

microfluidic networks
gel
fibrin
collagen
gelatin
channels

11.1 Introduction

This chapter focuses on techniques recently developed in our group to construct single channels and complex networks within gels of the extracellular matrix (ECM) proteins type I collagen and fibrin [1, 2]. It details methods for establishing and maintaining perfusion in these gels. The methods described here are suitable for forming networks of different sizes and shapes, and we provide illustrative examples.

Microfluidic networks, primarily in polydimethylsiloxane (PDMS) and silicon, are widely used in many applications, including disposable chips for medical diagnostics [3], microreactors for performing small-scale chemical synthesis [4], and devices for controlling cell behavior [5]. Only recently have these structures been extended to hydrogels [6–8], largely because gels are difficult to manipulate: Unlike PDMS and silicon, gels need to be hydrated to retain their shape, are often highly sensitive to temperature or pH, and can collapse irreversibly under mechanical agitation. ECM hydrogels are particularly fragile, and special care must be taken to avoid deforming any channels formed within them.

These materials complement previously described microfluidic gels comprising alginate or poly(ethylene glycol) [6, 8]. Because ECM is the natural substrate upon which cells adhere and spread, microfluidic ECM gels are well suited for cell culture. For instance, we have used ECM gels to form microvessels in vitro by seeding vascular cells in or on the gels [1, 9]. Microfluidic networks also enable fast convective delivery of fluids within hydrogels and allow spatial and temporal control of solute transport [10]. We have used our method for fabricating microfluidic networks to study diffusive and convective transport in ECM gels [2].

11.2 Materials

In our design, the gel lies on top of a supporting dish and is held in place by a PDMS housing. Channels in the gel are made by molding the gel around removable elements. If desired, addition of a lid and tubing enables perfusion under high flow and/or high pressure.

11.2.1 Supporting dishes

1. 100 mm–diameter tissue-culture polystyrene dishes (Corning)
2. No. 1.5 glass coverslips, 22 mm² (Corning)
3. Liquid PDMS (Sylgard 184, Dow Corning), freshly mixed as 1 part catalyst to 10 parts prepolymer and degassed in a vacuum

11.2.2 PDMS housing

1. *For single channels*
 - i. Silicon wafer (Montco Silicon) patterned with approximately 1 mm–thick negative photoresist (e.g., SU 8-50), using standard photolithographic techniques [see Figure 11.1(a) for pattern dimensions]

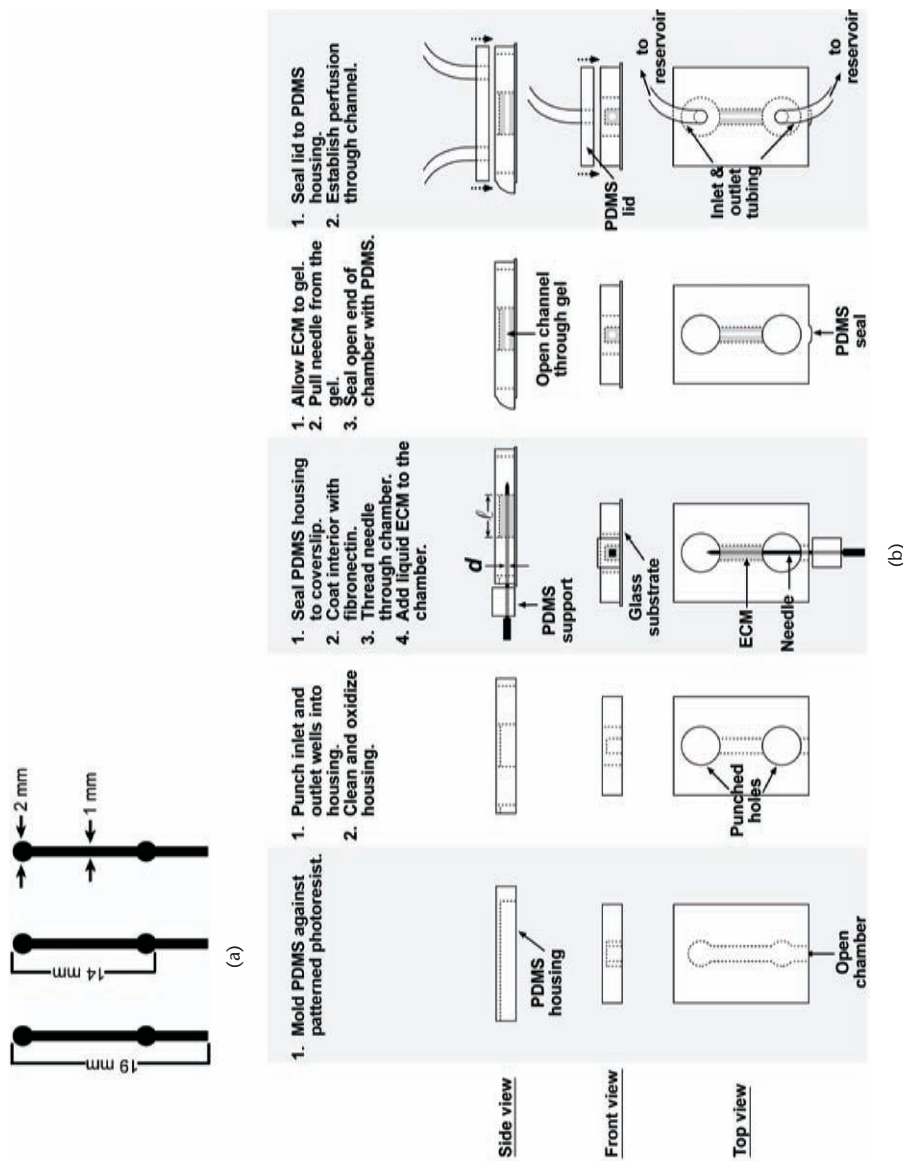


Figure 11.1 Forming ECM gels that contain single channels. (a) Lithography pattern for making the PDMS housing. The rounded features serve as guides for the hole punch. (b) Schematic for forming single channels by using a small-diameter needle as a removable element. The parameters d and l refer to the diameter and length of the channel, respectively. The last panel illustrates how to establish high-flow perfusion in the microfluidic gel; for further details or for perfusion under low flow, see Figure 11.3.

- ii. Single hole punch that produces circular or square holes (available at arts and crafts stores)
- iii. Liquid PDMS (see item 3 in Section 11.2.1)
2. *For networks*
 - i. Blocks of PDMS, approximately 1 mm and approximately 3 mm thick, cured at 60°C for several hours
 - ii. Hole punch

11.2.3 Removable elements (needles and gelatin mesh)

1. *For single channels*
 - i. Needles, 0.12 mm in diameter (Seirin J-type; Health Point Products)
 - ii. Liquid PDMS (see item 3 in Section 11.2.1)
 - iii. Sterile phosphate-buffered saline (PBS; Invitrogen)
 - iv. 0.1% w/v bovine serum albumin (BSA; Calbiochem) in PBS
2. *For networks*
 - i. Silicon wafer patterned with negative photoresist such that features have a rectangular cross section (50 to 100 μm thick) [see Figure 11.2(a) for pattern dimensions]
 - ii. Liquid PDMS (see item 3 in Section 11.2.1)
 - iii. 1% w/v Pluronic F127 (BASF) in PBS
 - iv. 10% w/v type A gelatin from pig skin (Sigma) in PBS, filter-sterilized at 60°C and stored as a gel at 4°C
 - v. 0.1% BSA and PBS

11.2.4 ECM proteins

1. *Collagen*
 - i. 8 to 10 mg/mL type I collagen from rat tail (BD Biosciences), stored at 4°C.
 - ii. 0.2M sodium hydroxide (NaOH; Sigma) in water, filter-sterilized.
 - iii. 7.5% w/v sterile sodium bicarbonate solution (NaHCO_3 ; Invitrogen).
 - iv. pH-indicator strips (Fisher Scientific), with a pH range of 5.0 to 10.0.
2. *Fibrin*
 - i. Human fibrinogen (Sigma), freshly reconstituted at 50 mg/mL in water. The solution is clarified at 16,000g for 1 minute before use. We use fibrinogen that contains approximately 60% clottable protein.
 - ii. Human thrombin (Sigma), reconstituted at 160 U/mL in water and stored at 4°C.
3. *Fibronectin*
 - i. Human fibronectin (BD Biosciences), reconstituted at 1 mg/mL in water and stored at 4°C. Avoid excessive mixing of the solution that may shear-polymerize the fibronectin.

11.2.5 High-flow perfusion

1. Plain silicon wafer
2. PE-50 polyethylene tubing (BD Biosciences)

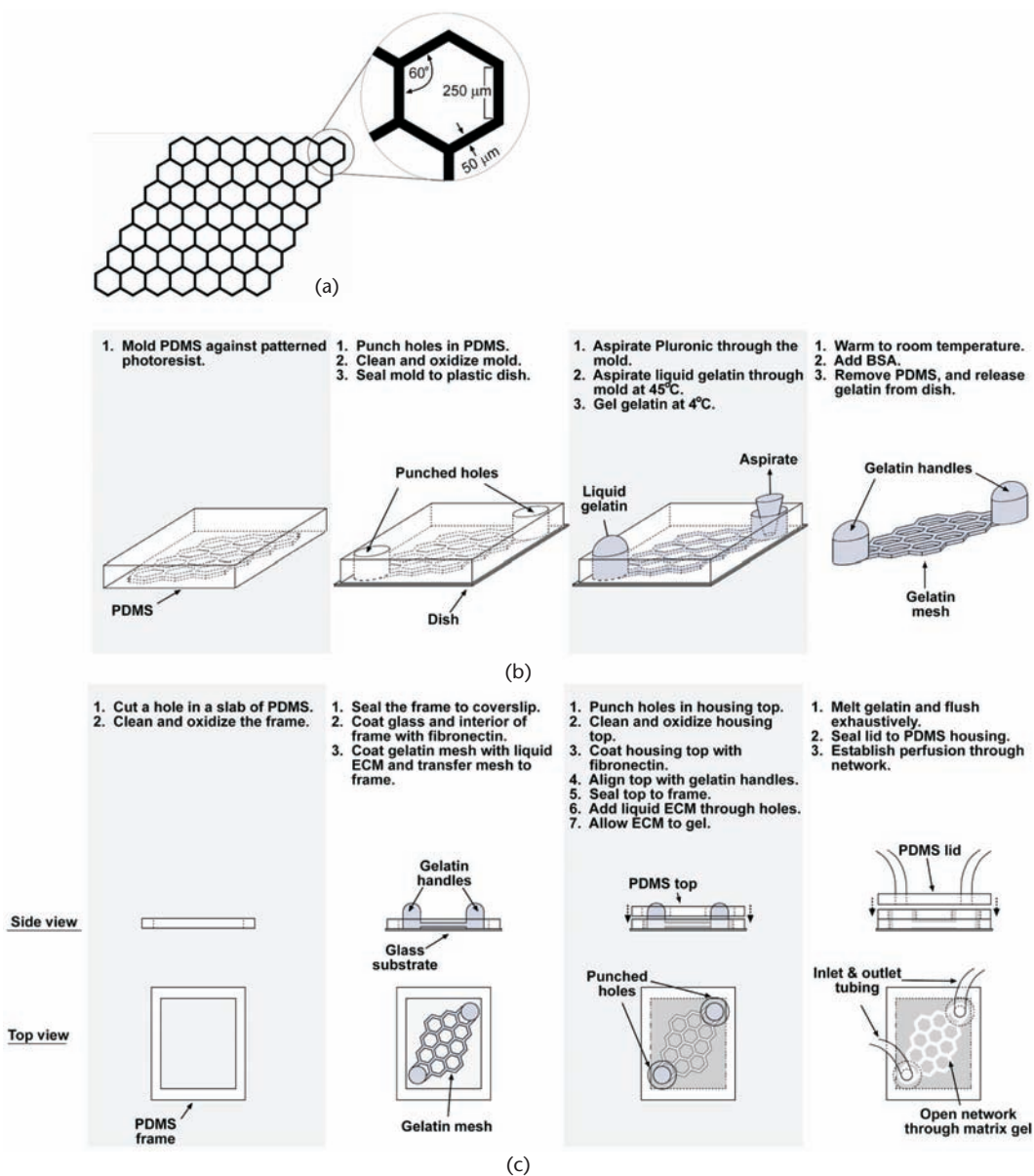


Figure 11.2 Forming ECM gels that contain networks. (a) Lithography pattern for a hexagonal network. (b) Schematic for molding gelatin meshes. (c) Schematic for forming networks within ECM gels by using the gelatin mesh as a removable element. The last panel illustrates how to establish high-flow perfusion in the microfluidic gel; for further details or for perfusion under low flow, see Figure 11.3.

- Liquid PDMS (see item 3 in Section 11.2.1)
- 100-mm-diameter deep petri dishes (Fisher Scientific)
- Glass slides, 1" \times 3" (Fisher Scientific)
- Small binder clips (Office Depot)

11.3 Methods

Both methods for creating microfluidic ECM gels are subtractive. In one method, partial encapsulation of a needle within liquid matrix, followed by manual removal of the needle after gelation, yields a single cylindrical channel. In the other method, partial encapsulation of a patterned gelatin mesh within liquid matrix, then removal of the gelatin after gelation by melting, yields an open network. In both cases, the channels are adjacent to inlet and outlet wells that enable perfusion of the system.

Surrounding each microfluidic gel is a PDMS housing, which serves as a mechanical support that is (nearly) impermeable to water. The system can be perfused under low flow rates and pressures by adding small volumes ($\sim 50 \mu\text{L}$) of perfusate to an open inlet and periodically removing liquid from the open outlet. Perfusion under high flow rates and/or pressures requires attaching a lid to the housing; this lid consists of a PDMS block threaded with two lengths of polyethylene tubing. The polyethylene tubing is, in turn, connected to inlet or outlet dishes of perfusate that serve as “reservoirs.” The relative heights of the dishes determine the pressure difference—and, indirectly, the flow rate—across the gel. Each gel lies on top of a glass coverslip for ease of visualization (e.g., with phase-contrast or fluorescence microscopy).

As a general rule, all cured PDMS, glass, and polyethylene tubing used for the system should be thoroughly cleaned and sterilized before use. Removal of dust that can cause leaks or clogs is critical. We usually sonicate each part in 70% ethanol for 1 minute or more, wash it with pure ethanol, and dry it with an aspirator. We use tweezers (as much as is practical) for handling and assemble the parts in a laminar flow hood.

The PDMS is replica-molded from a silicon master with features patterned in negative photoresist [11]. Other reviews provide details about photolithography used to fabricate these silicon masters [12]. Figures 11.1 and 11.2 show sample patterns that we have used in previous studies.

11.3.1 Construction of supporting dishes

1. Drill an approximately 1 cm^2 hole into the base of a tissue-culture polystyrene dish. All plastic debris should be removed manually under a stream of water. Sterilize the dish with pure ethanol, and allow it to dry.
2. Clean and dry a coverslip in a sterile hood.
3. Use a small amount of liquid PDMS to glue the coverslip over the hole in the dish, and cure. Additional coverslips may be used as spacers to tailor the height of the central coverslip.
4. Store the dish in a sterile hood.

11.3.2 Construction of PDMS housings

1. *For single channels*
 - i. Pour the PDMS into a petri dish that contains the patterned silicon master. The PDMS should flood the top of the photoresist by approximately 0.5 mm. Use a dish that is sufficiently larger than the silicon master to prevent a meniscus from forming near the edge of the pattern (e.g., a dish 150 mm in diameter is large enough for casting on 4" silicon wafers).

- ii. Cure, remove, and trim the PDMS into individual blocks, one feature per block.
 - iii. Use a hole punch to cut holes (~3 mm in diameter) at either end of the PDMS block, as shown in Figure 11.1(b). These holes will eventually form inlet and outlet wells next to a microfluidic gel.
 - iv. Sterilize the PDMS housing, and store it in a sterile hood.
2. *For networks*
- i. Cut an approximately 1 cm² rectangular hole from a 1-mm-thick PDMS block to create a “frame.”
 - ii. Use a hole punch to cut holes (~3 mm in diameter) near either corner of a 3-mm-thick PDMS block to create a “top,” as shown in Figure 11.2(c). The frame and top, when combined, will form the PDMS housing.
 - iii. Sterilize the frame and top, and store them in a sterile hood.

11.3.3 Preparation of removable elements

1. *For single channels*
- i. Deposit a thin stripe of liquid PDMS on a 100-mm-diameter petri dish, arrange 10 to 15 needles on the PDMS so that they lie parallel to the bottom of the dish, and cure the PDMS to fix the needles in place.
 - ii. Add PDMS to cover the needles, and cure.
 - iii. Cut out individual needles, and remove all PDMS from the needles, except for a rectangular block in front of the needle “handle,” as illustrated in Figure 11.1(b). This PDMS support serves as a mount to stabilize and suspend the needle.
 - iv. Sterilize the needle, and coat it with BSA for 40 minutes at room temperature (avoid exposing the PDMS support to BSA solution). Wash the needle exhaustively with water, and dry. The absorbed layer of BSA prevents ECM proteins from adhering to the needle [13].
2. *For networks*
- i. Cast PDMS on the patterned silicon wafer, and cure. The PDMS should flood the top of the photoresist by 3 to 5 mm.
 - ii. Cut the PDMS into individual molds, and use a hole punch to cut holes at either end of a PDMS mold, as shown in Figure 11.2(b).
 - iii. Clean the PDMS mold, oxidize it in a UV-ozone cleaner for 10 minutes, and place the patterned side down on a sterile petri dish.
 - iv. Aspirate a solution of Pluronic F127 through the PDMS network, and allow the Pluronic to absorb for 1 hour at room temperature. Absorbed Pluronic prevents ECM proteins from adhering to the mold [14]. Flush the network exhaustively with water after adsorption.
 - v. Warm the mold to 45°C, and aspirate liquid gelatin through the mold, as shown in Figure 11.2(b). Fill both holes in the PDMS mold with gelatin (after gelation, these gelatin cylinders will serve as handles for the mesh).
 - vi. Gel the gelatin at 4°C for 15 minutes or longer. Warm the gelatin to room temperature for 0.5 to 2 hours.
 - vii. Submerge the PDMS mold and gelatin in 0.1% BSA. Gently peel the PDMS mold from the dish, and release the gelatin from the mold and dish by repeatedly pipetting BSA solution onto the gelatin mesh.

viii. Store the gelatin mesh at 4°C in 0.1% BSA.

11.3.4 Formation of microfluidic gels

1. *Preparation of neutralized collagen solution*
 - i. Keep all components—dishes, PDMS housings, removable elements, reagents, mixing tubes, and so forth—at 4°C or on an ice bath.
 - ii. Prepare liquid collagen in this order: 12.5 parts 10× PBS, 1 part 7.5% NaHCO₃, approximately 12.5 parts 0.2M NaOH (the exact amount needed to neutralize collagen varies with the lot), and 100 parts collagen. Thoroughly mix the collagen without introducing bubbles into the mixture. Pipette collagen onto a pH-indicator strip—the pH should be 7.0 to 7.5.
2. *Preparation of liquid fibrinogen solution*
 - i. Mix fibrinogen solution with 1 to 5 U/mL of thrombin (see step 2 of Section 11.5.1). It is necessary to work quickly after adding thrombin since fibrinogen rapidly polymerizes to fibrin (in less than 5 minutes at room temperature).
3. *For single channels*
 - i. Oxidize the PDMS housing in a UV-ozone cleaner for 10 minutes. Place the PDMS housing patterned side down on the glass coverslip of the supporting dish.
 - ii. Coat the housing chamber with fibronectin (~10 μg/mL) for 40 minutes. Aspirate the chamber to dry.
 - iii. Carefully thread a needle into the PDMS housing, as in Figure 11.1(b). The PDMS support of the needle should firmly adhere to the dish, but the needle should be suspended above the dish.
 - iv. Prepare the liquid ECM (see step 1 or 2 of Section 11.3.4), and pipette approximately 10 μL into one of the wells in the housing. Tap the dish to force liquid ECM between the PDMS housing and glass coverslip, and aspirate excess ECM from the wells.
 - v. Add droplets of PBS to the tissue-culture dish (but *not* to the wells) to prevent dehydration.
 - vi. Gel the ECM on a water bath to evenly distribute heat and to buffer the system from vibrations. We typically gel collagen and fibrin at 22°C to 24°C for 2 hours.
 - vii. Add small drops of perfusate to the inlet and outlet wells, and make sure the liquid wicks along the side of the needle and contacts the ECM gel. Use two tweezers to remove the needle—one to hold the PDMS support and the other to withdraw the needle from the ECM. This process yields a single open channel within the ECM gel that is connected to two wells approximately 3 mm in diameter.
 - viii. Seal the open end of the chamber with liquid PDMS, fill the wells with perfusate, and cure the PDMS at 37°C.
4. *For networks*
 - i. Oxidize the PDMS frame and top in a UV-ozone cleaner for 10 minutes. Place the frame on the glass coverslip of the supporting dish.
 - ii. Coat the interior of the frame and top with fibronectin for 40 minutes. Aspirate to dry.

- iii. Prepare the liquid ECM (see step 1 or 2 of Section 11.3.4). Wash the gelatin mesh exhaustively with PBS, then one to two times with liquid ECM, and carefully transfer the mesh to the frame, as in Figure 11.2(c).
- iv. Align the holes of the PDMS top to the handles of the gelatin mesh, and place the PDMS top on the frame. Tap the top to ensure good contact with the frame.
- v. Fill the interior of the PDMS housing (the unit formed by the top and frame) with liquid ECM, leaving the upper portions of the gelatin mesh handles uncovered.
- vi. Add droplets of PBS to the dish to prevent dehydration.
- vii. Gel the ECM on a water bath to evenly distribute heat and to buffer the system from vibrations. We typically gel collagen and fibrin at 22°C to 24°C for 2 hours.
- viii. Heat the dish to 37°C to melt the gelatin, and exhaustively flush the system with perfusate to remove the gelatin. This process yields an approximately 1 cm² network within the ECM gel connected to two wells approximately 3 mm in diameter.

11.3.5 Perfusion of microfluidic gels

1. Dropwise perfusion

- i. Add perfusate to the inlet of the gel to establish flow, as shown in Figure 11.3(a). To maintain perfusion, regularly add perfusate to the inlet, and aspirate it from the outlet. Keep a small volume of perfusate in the outlet to promote flow.

2. High-flow perfusion

- i. Cut approximately 1-cm-long posts of PE-50 tubing, dip their ends in liquid PDMS, and stand them upright on a flat silicon wafer. The spacing of the tubing should roughly equal the spacing between the inlet and outlet wells of the PDMS housing. Cure the PDMS to fix the tubing in place.

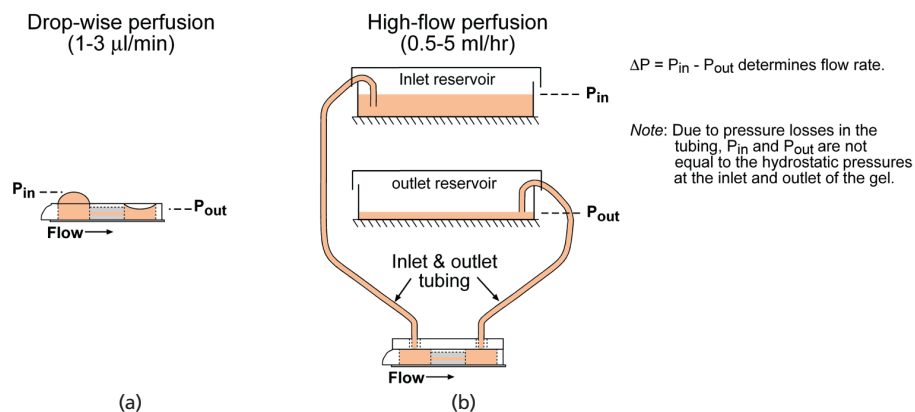


Figure 11.3 Perfusing microfluidic gels. (a) In dropwise perfusion, small drops ($\sim 20 \mu\text{L}$) of fluid are added to the inlet well of the system. Flow through the channel(s) occurs by the small pressure difference ($< 5 \text{ mm H}_2\text{O}$) between the inlet and outlet. To maintain continuous flow, additional media must be added at regular intervals to the inlet, while perfused media is removed from the outlet. (b) Under high-flow perfusion, a PDMS lid with tubing connects the microfluidic gel to inlet and outlet reservoirs of liquid held at hydrostatic pressures of P_{in} and P_{out} , respectively. The pressure difference between the two reservoirs drives perfusion. The hydrostatic pressures P_{in} and P_{out} of the reservoirs do not equal the pressures at the inlet and outlet of the gel due to pressure losses in the tubing.

- ii. Pour additional PDMS to cover the wafer (3 to 4 mm deep), but do not cover the top of the tubing. Cure and peel the PDMS from the silicon wafer, and remove the embedded tubing to yield PDMS with cylindrical holes. Trim the PDMS into blocks, two holes per block.
- iii. Cut 40 to 50 cm lengths of PE-50 tubing, and thread the tubing into the holes in the PDMS blocks. The mouth of the tubing should be level with the flat underside of the PDMS.
- iv. Apply a droplet of liquid PDMS to glue the tubing to the top side of the PDMS block, and cure. The liquid PDMS should wick completely around the tubing, but it should not wick down to the underside of the PDMS block.
- v. If desired, calibrate the fluidic resistance of the tubing (see step 3 of Section 11.5.2).
- vi. Sterilize the lid and attached tubing. Aspirate pure ethanol, then water, through the tubing. Aspirate to dry the inside of the tubing.
- vii. Place sterile glass slides in each of two deep petri dishes. Place the two free ends of the tubing in separate dishes, with each end under a glass slide.
- viii. Add perfusate to the inlet and outlet wells of the microfluidic gel, but do not fill the wells completely.
- ix. Fill the inlet reservoir dish with perfusate, introduce perfusate through the inlet tubing by vacuum, and clamp the tubing with a binder clip to stop flow. Aspirate excess liquid from the underside of the PDMS lid. Align the two openings in the lid with the inlet and outlet wells of the PDMS housing, and place the lid on the PDMS housing. Tap the lid to make a good seal with the housing.
- x. Unclamp the inlet tubing, and raise the inlet and outlet reservoirs, as illustrated in Figure 11.3(b). The perfusate should flow into the outlet tubing within a few minutes.

11.4 Anticipated Results

Figure 11.4 shows typical results from both methods. By light microscopy, the fibers of the gels are uniformly distributed and randomly oriented. Walls of the channels are clearly defined within the surrounding matrix. Single channels have circular cross sections, with diameters corresponding to the diameters of the needles [Figure 11.4(a), inset], while network channels have rectangular cross sections that reflect the shapes created by lithography [Figure 11.4(b), inset]. Perfusion with a suspension of labeled beads leads to flow with low velocities at the walls of the channels and high velocities along the center of the flowing stream (Figure 11.4).

11.5 Application Notes

11.5.1 Rate of gelation

1. For collagen gels, the rate of gelation largely dictates the resulting porosity. Increasing temperature (up to 37°C) or pH (up to approximately 8.0) increases the rate of gelation and yields gels with fine, faint fibrils [15, 16]. These gels are less

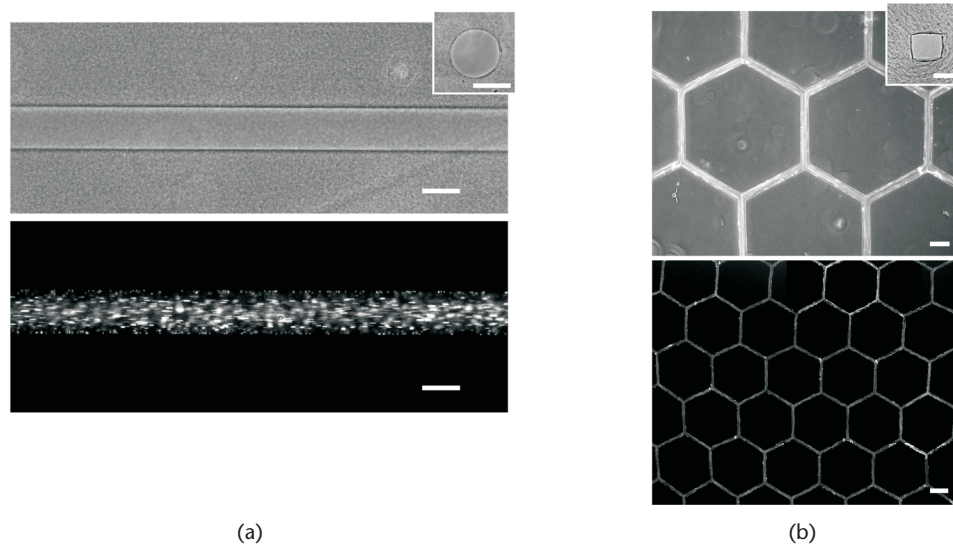


Figure 11.4 Phase and fluorescence images of microfluidic channels. (a) Phase-contrast image of a single 120- μm -diameter channel in collagen (top panel) and a fluorescence image of 1- μm -diameter beads perfusing the channel (bottom panel). Inset: cross section of a single channel in collagen. (Reprinted from [1] with permission from Elsevier.) (b) Phase-contrast image of a hexagonal network in fibrin (top panel) (reprinted from [2] with permission from the Royal Society of Chemistry) and a collage of fluorescence images of a single hexagonal network in fibrin (bottom panel). Channels are 60- μm -wide and perfused with fluorescently labeled 1- μm -diameter beads. Inset: cross section of a 100- μm -wide channel in collagen. Scale bars refer to 400 μm in the bottom panel of (b) and to 100 μm elsewhere.

porous and stiff than those prepared at lower temperatures or pH. In our studies, we primarily use gelation temperatures of 22°C to 24°C and pH of 7.0 to 7.5.

2. For fibrin gels, increasing the concentration of thrombin yields compact gels with smaller fibers and pores [17, 18].

11.5.2 Resistance of microfluidic gels and tubing

The flow rate through a microfluidic gel is the sum of the flow rates through the channels and through the gel (i.e., interstitial flow). Flow through channels is normally orders of magnitude larger than interstitial flow, but this rule may not hold for gels that contain channels on the order of 10 μm in width or for gels of large cross-sectional area. Flow through channels is inversely related to fluidic resistance, which depends on the lengths and cross-sectional areas of the channels.

1. Dropwise perfusion with PBS of a channel that is 6 mm in length and 120 μm in diameter yields flow rates of 1 to 3 $\mu\text{L}/\text{min}$. If the flow into the outlet well is not noticeable after a few minutes of perfusion, a constriction or clog likely exists at one end of the channel.
2. For the same channel dimensions and perfusate as above, perfusion under a pressure difference of 6 cm H_2O between the inlet and outlet reservoirs yields flow rates of 1.2 to 1.5 mL/hr.

3. The tubing of the perfusion lid is a source of variation in perfusion rates between samples. Equal lengths of polyethylene tubing often do not have the same fluidic resistance. We always measure the resistance of each segment of tubing before use and alter it as needed by trimming the tubing. We normally adjust each tubing in a lid to have a resistance of 26.4 cm H₂O·min/mL at room temperature with PBS as perfusate.
4. In both perfusion systems depicted in Figure 11.3, the flow rate decreases over time as the difference in fluid height in the inlets and outlets decreases. Using reservoirs with a large surface area can lessen this effect.

11.6 Discussion and Commentary

The most common problems we have encountered are deformation of the ECM gels and development of leaks (see Troubleshooting Table). Contaminants can be avoided with standard sterile technique or by assembling parts in a clean room environment.

11.6.1 Enlarged and/or deformed channels

1. Single channels can easily be widened or deformed if the needle is haphazardly removed from the gel. The PDMS support should be held firmly in place with tweezers while the needle is removed, without applying excessive force that bends the needle. During gelation, floating the system on a water bath helps minimize any vibrations.
2. The gelatin mesh swells if it is released from the PDMS mold without first equilibrating to room temperature for at least 30 minutes. During gelation of ECM, swelling leads to enlarged and deformed channels in the microfluidic gel.
3. The gels deform if bubbles enter the channels or if the gelatin mesh has bubbles. Bubbles arise when insufficient liquid is present at the inlet or outlet or when the gel dries. We always make sure the gels are hydrated at their ends. Bubbles form during micromolding of gelatin if the PDMS mold becomes dehydrated.
4. Gelatin meshes, especially thin ones, are very flexible. We use minutien pins to maneuver the meshes and to stabilize the mesh during addition of ECM.
5. The basement membrane extract Matrigel (BD Biosciences) behaves strangely in our system. It swells significantly during perfusion, and channels often pinch off completely over several hours. We recommend using Matrigel only as a dilute mixture with collagen.

11.6.2 Leaks between the gel and PDMS or between the gel and coverslip

1. Leaks can form if the coverslip or PDMS is poorly coated with fibronectin. We always oxidize PDMS housings in a UV-ozone cleaner for at least 10 minutes to enhance the absorption of fibronectin onto the PDMS surface.
2. Mechanical stress can loosen the bond between the gel and surrounding surfaces. Excessive force when connecting the perfusion lid to the PDMS housing will cause the gel to detach from the housing.
3. Trace amounts of BSA from a gelatin mesh can inhibit the adhesion of the gel to surrounding surfaces. We typically wash meshes with PBS to remove BSA.

Troubleshooting Table

Problem	Potential Cause	Solution
Deformation of single channel	Vibration of needle during gelation; vibration of needle during removal	Gel ECM on water bath. Firmly hold PDMS support during removal of needle.
Deformation of networks	Swelling of gelatin mesh	Equilibrate gelatin mesh at room temperature before releasing from mold.
Air bubbles in channels or networks	Insufficient liquid at inlet or outlet of gel	Ensure inlets and outlets are well-hydrated.
Leak between gel and housing	Weak adhesion between gel and housing	Oxidize PDMS housing and increase time for fibronectin absorption. Avoid applying mechanical stress to housing after ECM has gelled.
Leak between housing and perfusion lid	Liquid trapped between housing and lid	Dry surfaces of housing and lid before placing them together.

- Both the lid and housing must be dry to make a firm seal. Any liquid, especially culture media, trapped between the PDMS surfaces will weaken the seal. Catastrophic failure can then occur if the system is placed under high hydrostatic pressures (10 to 30 cm H₂O).

11.7 Summary Points

- Gelation around needles and their subsequent removal can yield single channels with round cross sections of 50 μm or more in diameter.
- Gelation around gelatin meshes and their subsequent melting can yield networks with rectangular cross sections of 6 μm or more in width.
- Perfusion of microfluidic gels takes place by introducing drops of media to the inlets of the gels (low flow) or by applying a perfusion lid and pressure difference to the gel housing (high flow).
- Maintenance of flow requires adequate adhesion between gel and surrounding surfaces and elimination of debris that can clog inlets.

Acknowledgments

We thank Andrew Golden for providing images of fibrin networks. This work was supported by the National Institute of Biomedical Imaging and Bioengineering (award EB005792).

References

- [1] Chrobak, K. M., Potter, D. R., and Tien, J., "Formation of perfused, functional microvascular tubes in vitro," *Microvascular Research*, Vol. 71, No. 3, 2006, pp. 185–196.
- [2] Golden, A. P., and Tien, J., "Fabrication of microfluidic hydrogels using molded gelatin as a sacrificial element," *Lab on a Chip*, Vol. 7, No. 6, 2007, pp. 720–725.
- [3] Yager, P., et al., "Microfluidic diagnostic technologies for global public health," *Nature*, Vol. 442, No. 7101, 2006, pp. 412–418.
- [4] Lee, C. C., et al., "Multistep synthesis of a radiolabeled imaging probe using integrated microfluidics," *Science*, Vol. 310, No. 5755, 2005, pp. 1793–1796.
- [5] Whitesides, G. M., "The origins and the future of microfluidics," *Nature*, Vol. 442, No. 7101, 2006, pp. 368–373.

Subtractive Methods for Forming Microfluidic Gels of Extracellular Matrix Proteins

- [6] Cabodi, M., et al., "A microfluidic biomaterial," *J. American Chemical Society*, Vol. 127, No. 40, 2005, pp. 13788–13789.
- [7] Vernon, R. B., et al., "Native fibrillar collagen membranes of micron-scale and submicron thicknesses for cell support and perfusion," *Biomaterials*, Vol. 26, No. 10, 2005, pp. 1109–1117.
- [8] Hahn, M. S., Miller, J. S., and West, J. L., "Three-dimensional biochemical and biomechanical patterning of hydrogels for guiding cell behavior," *Advanced Materials*, Vol. 18, No. 20, 2006, pp. 2679–2684.
- [9] Price, G. M., Chrobak, K. M., and Tien, J., "Effect of cyclic AMP on barrier function of human lymphatic microvascular tubes," *Microvascular Research*, Vol. 76, No. 1, 2008, pp. 46–51.
- [10] Choi, N. W., et al., "Microfluidic scaffolds for tissue engineering," *Nature Materials*, Vol. 6, No. 11, 2007, pp. 908–915.
- [11] Whitesides, G. M., et al., "Soft lithography in biology and biochemistry," *Annual Review of Biomedical Engineering*, Vol. 3, 2001, pp. 335–373.
- [12] Tien, J., and Chen, C. S., "Microarrays of cells," in A. Atala and R. Lanza, (eds.), *Methods of Tissue Engineering*, San Diego, CA: Academic Press, 2001, pp. 113–120.
- [13] Tang, M. D., Golden, A. P., and Tien, J., "Molding of three-dimensional microstructures of gels," *J. American Chemical Society*, Vol. 125, No. 43, 2003, pp. 12988–12989.
- [14] Tan, J. L., et al., "Simple approach to micropattern cells on common culture substrates by tuning substrate wettability," *Tissue Engineering*, Vol. 10, Nos. 5–6, 2004, pp. 865–872.
- [15] Wood, G. C., and Keech, M. K., "The formation of fibrils from collagen solutions. 1. The effect of experimental conditions: Kinetic and electron-microscope studies," *Biochemical Journal*, Vol. 75, No. 3, 1960, pp. 588–598.
- [16] Williams, B. R., et al., "Collagen fibril formation: Optimal in vitro conditions and preliminary kinetic results," *J. Biol. Chem.*, Vol. 253, No. 18, 1978, pp. 6578–6585.
- [17] Blombäck, B., et al., "Native fibrin gel networks observed by 3D microscopy, permeation and turbidity," *Biochimica et Biophysica Acta*, Vol. 997, No. 1–2, 1989, pp. 96–110.
- [18] Blombäck, B., et al., "Fibrin in human plasma: Gel architectures governed by rate and nature of fibrinogen activation," *Thrombosis Research*, Vol. 75, No. 5, 1994, pp. 521–538.