Fabrication of microfluidic hydrogels using molded gelatin as a sacrificial element†

Andrew P. Golden and Joe Tien*

Received 18th December 2006, Accepted 1st March 2007
First published as an Advance Article on the web 21st March 2007
DOI: 10.1039/b618409j

This paper describes a general procedure for the formation of hydrogels that contain microfluidic networks. In this procedure, micromolded meshes of gelatin served as sacrificial materials. Encapsulation of gelatin meshes in a hydrogel and subsequent melting and flushing of the gelatin left behind interconnected channels in the hydrogel. The channels were as narrow as ~6 μm, and faithfully replicated the features in the original gelatin mesh. Fifty micrometre wide microfluidic networks in collagen and fibrin readily enabled delivery of macromolecules and particles into the channels and transport of macromolecules from channels into the bulk of the gels. Microfluidic gels were also suitable as scaffolds for cell culture, and could be seeded by human microvascular endothelial cells to form rudimentary endothelial networks for potential use in tissue engineering.

Introduction

Many proposed applications in tissue engineering and drug delivery require the rapid transport and exchange of materials through hydrogels.[1] One strategy to control the rate of transport in a gel is to alter the densities and geometries of pores in the gel.[2] Application of a pressure difference across the ends of a gel[3–5] induces transport *via* diffusion and convection (here, 'convection' refers to transport of solute that is carried by a flowing fluid). This process can deliver enough solutes to the interior of a gel to sustain the metabolism of embedded cells.[6,7] The large hydraulic resistance of bulk gels typically requires the use of substantial driving pressure differences to induce physiologically useful flow rates.

Recently, we[8,9] and others[10–13] have proposed the use of 'microfluidic' hydrogels as scaffolds for the transport of materials. These gels contain interconnected channels that have widths of 10–1000 μm. The major advantage of this class of gels is that the large size of these channels (compared to the sub-μm size of pores) greatly reduces the fluidic resistance of the gels. Transport of solute in these open gels requires convective delivery through a series of channels and subsequent diffusion and convection through the pores of the gel. For a desired transport rate, microfluidic gels require a much smaller driving pressure difference than bulk gels do. To date, microfluidic channels have been formed by lithographic processing of natural materials, such as alginate,[10] and synthetic ones, such as gels made from photopolymerized poly(ethylene glycol).[12] Extracellular matrix (ECM) gels serve as natural cell scaffolds (*e.g.* for microvascular tissue engineering), but methods for forming channels in ECM gels, such as native collagen, are currently limited to simple geometries, such as arrays of cylindrical tubes.[9,11]

We have previously described a sacrificial strategy, based on selective digestion of Matrigel (a gel of basement membrane proteins), to form cavities in collagen gels.[14] In theory, this procedure could be extended to form open networks in a gel, but we have found it difficult to implement this idea: Matrigel is brittle when molded into microstructures with large aspect ratios, and tends to fracture upon manipulation.

Our current work introduces a general procedure for the formation of microfluidic gels, with an emphasis on gels of native ECM proteins, such as type I collagen and fibrin. The basis of this method is the encapsulation of micromolded meshes of gelatin in a second gel and removal of gelatin by heating and flushing to leave interconnected channels in the remaining gel. Because gelatin is highly elastic and mechanically robust, we expected it to withstand the manipulation required for micromolding and encapsulation. We used soft lithography[15] to form the gelatin meshes with features as fine as 6 μm. Replication in a second gel formed channels with the same resolution; these channels could withstand intralumenal pressures of ~80 cm H_2O without fracture.

This work also examines the functional properties of microfluidic gels: We determined the transport properties of these gels by analyzing the delivery of two representative solutes—rhodamine B and bovine serum albumin (BSA)—in microfluidic collagen gels, and found that transport had both a diffusive and convective component under the conditions used. To assay the compatibility of this gelatin-based procedure with cell culture, we examined the viability of fibroblasts embedded in microfluidic collagen gels. We also demonstrated that microvascular endothelial cells attached, spread, and proliferated on the channels in these gels to form rudimentary endothelial networks that could be perfused.

Department of Biomedical Engineering, Boston University, 44 Cummington Street, Boston, MA 02215, USA. E-mail: jtien@bu.edu.; Fax: +1 (617) 353-6766; Tel: +1 (617) 358-3055
† This paper is part of a special issue 'Cell and Tissue Engineering in Microsystems' with guest editors Sangeeta Bhatia (MIT) and Christopher Chen (University of Pennsylvania).

Materials and methods

Materials

Polydimethylsiloxane (PDMS) was obtained as Sylgard 184 from Dow Corning, and was used at the standard formulation of 1 part catalyst to 10 parts base. Bovine serum albumin (BSA) was purchased from Calbiochem. Pluronic F127, a copolymer of ethylene oxide and propylene oxide, was a gift from BASF. Type I collagen from rat tail (6–8 mg mL^{-1}), Matrigel (10 mg mL^{-1}), and human fibronectin were obtained from BD Biosciences. Sterile phosphate-buffered saline (PBS), cell culture supplements, and Alexa Fluor 488-labeled BSA were purchased from Invitrogen. PolyFluor 570-labeled microspheres (1 μm diameter) were purchased from Polysciences. Human dermal fibroblasts and human dermal microvascular endothelial cells (HDMECs) were originally obtained as frozen suspensions from Cambrex. All other materials were purchased from Sigma-Aldrich. All solutions were prepared and used under sterile conditions.

Formation of micromolded meshes of gelatin

Gelatin meshes were formed by micromolding in microfluidic networks.[16] Microfluidic networks consisted of patterned PDMS stamps passively adherent to an underlying flat substrate (glass or pre-oxidized PDMS). PDMS stamps were cast against lithographically patterned masters, sterilized with ethanol, and lightly oxidized in UV/ozone ($\leqslant$15 min) before use. Networks were treated with Pluronic F127 (1–6% in PBS, 1 h) to render them non-adherent to gels,[17,18] before introduction of liquid gelatin at 45 °C (10% in PBS, type A from pig skin) and gelation at 4 °C for $\geqslant$15 min. In some samples, the gelatin solution was doped with Oregon Green 488-conjugated gelatin (3 mg mL^{-1}, Molecular Probes) before use. All samples were warmed to 25 °C for 0.5–2 h and then demolded in 1% BSA by separating the stamp from the substrate with tweezers and gently pipetting fluid onto the meshes.

Formation of microfluidic gels

To form a microfluidic gel, a gelatin mesh was transferred under 1% BSA to a fibronectin-coated well (5–10 mm^2 area, 0.2–1 mm depth) in PDMS, and washed with liquid gel precursor while making sure the mesh remained completely immersed except at its ends. A lid of PDMS with two ~5 mm diameter holes was carefully placed over the mesh and liquid precursor so that the holes lay on opposite ends of the mesh. Gel precursors were: type I collagen from rat tail, neutralized on ice with 10× PBS, 7.5% $NaHCO_3$, and 0.2 M NaOH; Matrigel, used as is; and fibrinogen, mixed at 50 mg mL^{-1} with 3.4 U mL^{-1} thrombin just before use. After gelation of the precursor at room temperature for 1 h, heating to 37 °C melted the gelatin, which was removed by exhaustively flushing the gel with PBS or 1% BSA through the two holes in the PDMS lid. This procedure yielded a microfluidic gel, held in place by PDMS, with one hole at each end for introduction of a perfusate. For long-term perfusion experiments, the two holes were connected to reservoirs of perfusate by PE-50 polyethylene tubing (exactly as described in Chrobak *et al.*[9]).

Gels that contained two layers of microfluidic networks were formed similarly, except two meshes (oriented perpendicularly) were used. Composite gels, in which the microfluidic networks were bounded by collagen and fibrin, were formed by placing gelatin meshes on pre-gelled collagen (or fibrin), encasing in liquid precursor of fibrin (or collagen), and melting the gelatin.

Microscopy and histology

All images were taken on a Zeiss Axiovert 200 M inverted microscope equipped with Plan-Neo 5×/0.15 and 10×/0.30 objectives and Axiocam HRm and MRm cameras. To visualize sections of microfluidic networks, we fixed gels with 4% paraformaldehyde or 1% glutaraldehyde for ~4 h and washed with PBS. Samples were then dehydrated in increasing concentrations of poly(ethylene glycol) (MW 1500) at 60 °C, cooled to 25 °C, cut into ~10 μm thick sections on a microtome, and imaged by phase-contrast microscopy. This method of sectioning preserves the cross-sectional shape of gels better than paraffin-based or frozen sectioning does.[19,20]

Transport in microfluidic gels

A solution of Alexa Fluor 488-labeled BSA (20 μg mL^{-1} in 4% unlabeled BSA) or Rhodamine B (1 μg mL^{-1} in 4% unlabeled BSA) was flowed through 50 μm wide hexagonal microfluidic networks (periodicity = 750 μm) in collagen. Inlet pressures were held at 1 cm H_2O, while outlet pressures were varied to obtain desired pressure differences across the networks. Fluorescence images for data presented in Fig. 4 (see later) were taken every 30 s, integrated over circular regions (radius = 100 μm) that were ~5 mm from the inlet and ~75 μm downstream of branch-points. Transport rates were estimated by calculating the change in integrated fluorescence intensity with respect to time (dI/dt) during the initial 5 min of fluorophore introduction, and by assuming that fluorescence intensity was proportional to the amount of fluorophore. The time at which fluorophore was introduced was taken to be $t = 0$.

Cell culture in microfluidic gels

Human dermal fibroblasts were routinely cultured in Dulbecco's Modified Eagle's Medium (DMEM) supplemented with 10% fetal bovine serum (FBS), 2 mM L-glutamine, 100 U mL^{-1} penicillin, and 100 μg mL^{-1} streptomycin (1% GPS). Fibroblasts were suspended at ~10^5 cells mL^{-1} in cold, neutralized collagen solution. The suspension was gelled around a gelatin mesh at room temperature for 15 min and then placed at 37 °C and flushed with culture media. Hoechst 33342 (1 μg mL^{-1}) and propidium iodide (2 μg mL^{-1}) were introduced by adding ~100 μL of labeled media to one end of the network and allowing the mixture to perfuse the gel for 30 min.

HDMECs were routinely cultured on gelatin-coated dishes in MCDB131 supplemented with 10% FBS, 1 μg mL^{-1} hydrocortisone, 80 μM dibutyryl cyclic AMP, 25 μg mL^{-1} endothelial cell growth supplement, 2 U mL^{-1} heparin, 0.2 mM ascorbic acid 2-phosphate, and 1% GPS. HDMECs

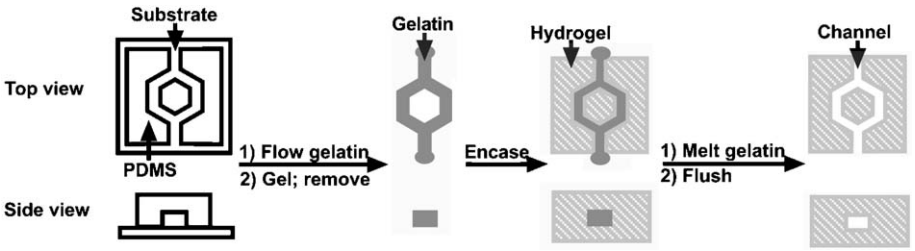

Fig. 1 Schematic diagram of fabrication of microfluidic gels. Sealing a PDMS stamp to a substrate (glass or pre-oxidized PDMS) created a microfluidic network. Sequential introduction of Pluronic (1–6% in PBS) and liquid gelatin into the channels, and gelation at 4 °C for 15 min and at 23 °C for 2 h, yielded a gelatin mesh that easily separated from the channels. Encapsulation of the mesh in a liquid hydrogel precursor (type I collagen, fibrinogen, Matrigel), polymerization of the precursor, and flushing at 37 °C, yielded a hydrogel with open microchannels.

were seeded into channels of an open collagen gel as a suspension of $\sim 10^8$ cells mL^{-1}; non-adherent cells were removed after <5 min by flushing with culture media. Flow in endothelialized networks was typically maintained for several days through polyethylene tubing at an inlet pressure of 5 cm H_2O and an outlet pressure of 0 cm H_2O. At these pressures, 99% of the flow was through channels and not through bulk gel.

Results and discussion

Formation of micromolded meshes of gelatin

The sacrificial elements in this work were micromolded gelatin meshes. To form them, we first sealed oxidized PDMS stamps to flat substrates (glass or PDMS) and absorbed an ethylene oxide–propylene oxide–ethylene oxide copolymer (Pluronic) in the resulting channels (Fig. 1). We then introduced a concentrated solution ($\sim$10%) of gelatin into the channels and gelled it by cooling. The absorbed layer of Pluronic prevented adhesion of gelatin to the channels.[18] Removal of the stamp and gentle agitation released the gelatin as a sturdy, flexible free-standing mesh (Fig. 2A). We have made gelatin meshes as large as 85 cm^2 (with 50 μm sized features) or with features as narrow as 6 μm by using vacuum to enhance the filling of channels by the viscous solution of gelatin.[21]

Although the proportions of the meshes mimicked those of features on the PDMS stamps, we noticed that the gelatin consistently swelled after release from underlying substrates. Gels swelled most rapidly within 10 min after release, and slowly for another 60 min (Fig. 2B). The time at which the gelatin was held at room temperature before release affected the degree of swelling, with longer times resulting in less swelling. Under optimal conditions (23 °C for 30 min before release), the meshes swelled by 18.7% $\pm$ 0.1% after $\sim$1 h in saline (Fig. 2B). The residual swelling is most likely an inherent property of gelatin.[22–24]

Formation of microfluidic gels

Encasement of a gelatin mesh in a liquid gel precursor (except at the ends of the mesh), gelation of the precursor at room temperature, and heating to 37 °C yielded an open network of channels throughout the hydrogel. Because the two ends of the mesh were not encased, they melted to yield openings at either end of the gel; extensive flushing with saline or 1% BSA through these openings removed the liquid gelatin. To investigate whether removal of gelatin was quantitative, we used fluorescently-labeled gelatin (Oregon Green 488, Molecular Probes) and recorded the residual fluorescence intensity after flushing; the intensity within the channels was indistinguishable from the background intensity of the surrounding collagen.

Fig. 3A shows an example of a microfluidic collagen gel that contained $\sim$50 μm wide channels. The features are as well-defined as those on the original PDMS stamp, indicating that the pore size of the gelatin was small enough to prevent monomeric collagen from infiltrating the gelatin before polymerization. Histological sections indicated that the channel walls preserved their rectangular cross-sectional profile (Fig. 3A, inset); the lithographic procedure used to create the

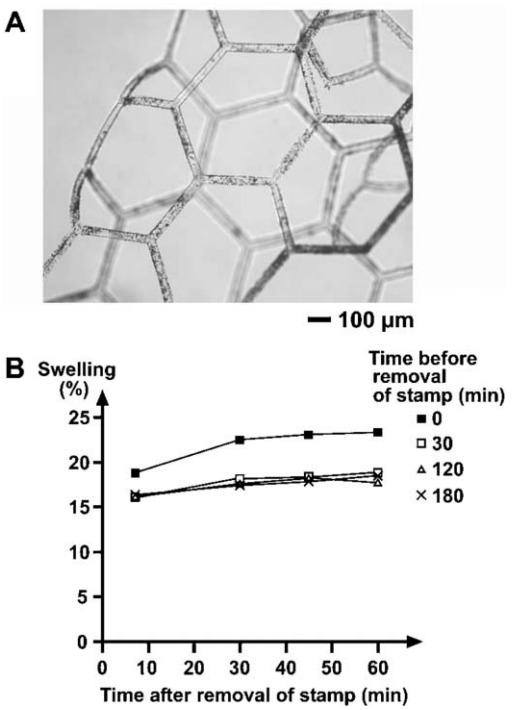

Fig. 2 Formation of gelatin meshes. (A) Phase-contrast image of a mesh decorated with iron powder ($\sim$4 μm sized) for visualization. (B) Plots of swelling of gelatin meshes as a function of time. The meshes were gelled at 4 °C for 15 min, warmed up to 23 °C for 0, 30, 120, and 180 min, removed from the stamp and substrate, and observed for an additional 60 min. Errors were less than <1% swelling.

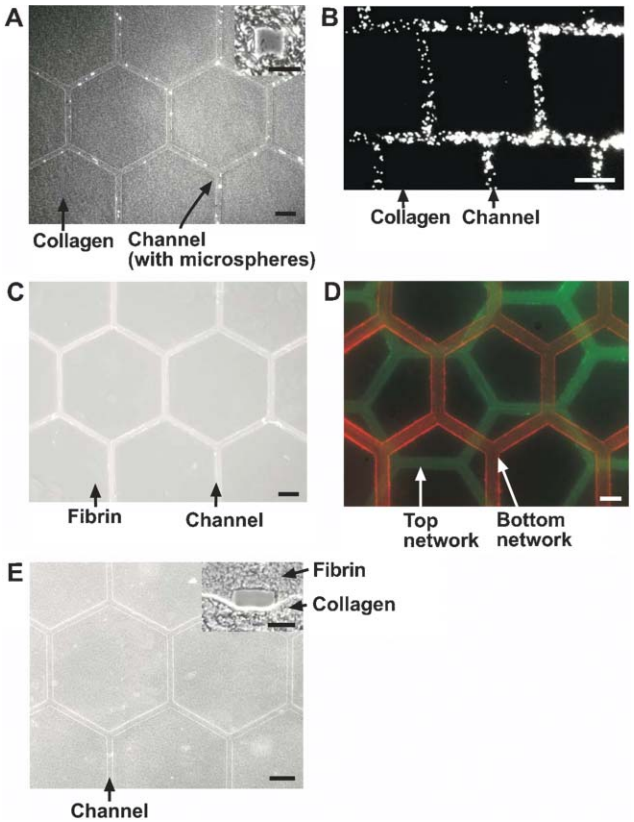

Fig. 3 Images of microfluidic gels. (A) Overlaid phase-contrast and fluorescence images of a hexagonal network in collagen perfused with 1 μm diameter fluorescent microspheres. Inset, an image of a cross-section of a collagen channel. (B) Fluorescence image of 6 μm wide channels in collagen perfused with microspheres. (C) Overlaid phase-contrast and fluorescence images of a hexagonal network in fibrin gel perfused with microspheres. (D) Collagen gels with multiplanar networks. The top and bottom networks were perfused by suspensions of green and red fluorescent beads, respectively. The image represents an overlay of images captured at the planes of each network (networks separated by ~400 μm). (E) An open network formed between a composite of collagen and fibrin. Inset, cross-sectional view. Scale bars refer to 200 μm (A, C–E), 25 μm (B), and 50 μm (insets).

PDMS stamp generates features with these cross-sectional shapes. Placement of a liquid solution at one end of the network resulted in rapid flow of the solution through the channels of the gel, as shown by the delivery of 1 μm diameter fluorescent microspheres by convection (Fig. 3A). Flow was slower, but still present, in microfluidic collagen gels with 6 μm-wide channels (Fig. 3B). This procedure formed microfluidic networks in fibrin (Fig. 3C) and Matrigel, although, surprisingly, the features in Matrigel narrowed over time. As expected, co-encapsulation and melting of two gelatin meshes yielded microfluidic gels that contained two independent networks (Fig. 3D).

By using pre-gelled layers as a substrate, this approach can also form microfluidic networks in composite gels that consist of two different chemistries. For example, sandwiching a gelatin mesh between a pre-gelled layer of collagen and liquid fibrinogen and subsequent gelling, melting, and flushing yielded an open composite whose channels were bounded by both collagen and fibrin (Fig. 3E). Histological sections confirmed that the patterns of these composite microstructures remained faithful to the original microfluidic molds (Fig. 3E, inset). Despite the difference in compositions of the two halves, the channels adhered strongly enough to confine perfused microspheres. We suspect that entanglement of polymeric chains at the interface may have enhanced their adhesion.

To test the mechanical strength of microfluidic gels, we subjected them to lumenal pressures of up to 80 cm H_2O while simultaneously flowing a suspension of fluorescent microspheres through them. For these tests, we used a PDMS lid with a hole so that the gel was directly exposed to atmospheric pressure at one face. Under these conditions, we observed substantial interstitial delivery of water from the channels to the surface of the exposed gel, but never observed fracturing of the channels (indicated by massive leaking of microspheres). These histological and mechanical tests thus indicate that the microfluidic gels are monolithic.

Transport in microfluidic gels

To determine whether microfluidic networks increase the rate of transport into these gels, we perfused them with a solution of rhodamine, and compared the delivery of fluorophore with that in bulk gels (Fig. 4A). Our measurements of fluorescence intensities as a function of time demonstrated that transport of materials to a gel was enhanced by the presence of microfluidic networks. In the absence of channels, transport was essentially driven by interstitial convection: The diffusion coefficient of rhodamine in aqueous solutions is $\sim 10^3$ μm^2 s^{-1}, so transport across the 5 mm extent of the gel by diffusion would take place over several hours. Under the pressure drop used (8 mm H_2O over 5 mm extent of gel), interstitial flow was $\leqslant 0.2$ mm min^{-1} and transported rhodamine was undetectable for the initial ~20 min. In the presence of channels, transport occurred by both convection and diffusion: Like the microspheres shown in Fig. 3, rhodamine was delivered by convection into channels. In contrast to microspheres, however, the rhodamine was readily delivered by diffusion and convection into the gel from channels within 1 min (Fig. 4A). Thus, the presence of microfluidic channels greatly enhanced the rate of transport into gels.

We also investigated whether the rate of transport was dependent on flow rate. Fig. 4B shows fluorescence intensity profiles from a representative microfluidic gel after sequential introduction and removal of a solution of rhodamine at three pressure differences (and flow rates): 8 mm H_2O (~48 μL h^{-1}), 30 mm H_2O (~141 μL h^{-1}), and 50 mm H_2O (~292 μL h^{-1}). Each such plot yielded six transport rates (three for delivery of solute, three for extraction). These rates are plotted in Fig. 4C for transport of rhodamine and of fluorescent BSA. As expected, the transport rate increased with an increase in flow rate or a decrease in the molecular weight of the perfusant (molecular weights of rhodamine and BSA are 479 and ~67000, respectively). We did not find that transport rate reached a limiting value.

Use of microfluidic gels as scaffolds for tissue engineering

To demonstrate the compatibility of these gels with cell culture (*e.g.*, to show that exposure to soluble gelatin is not cytotoxic), we examined the viability of human fibroblasts embedded

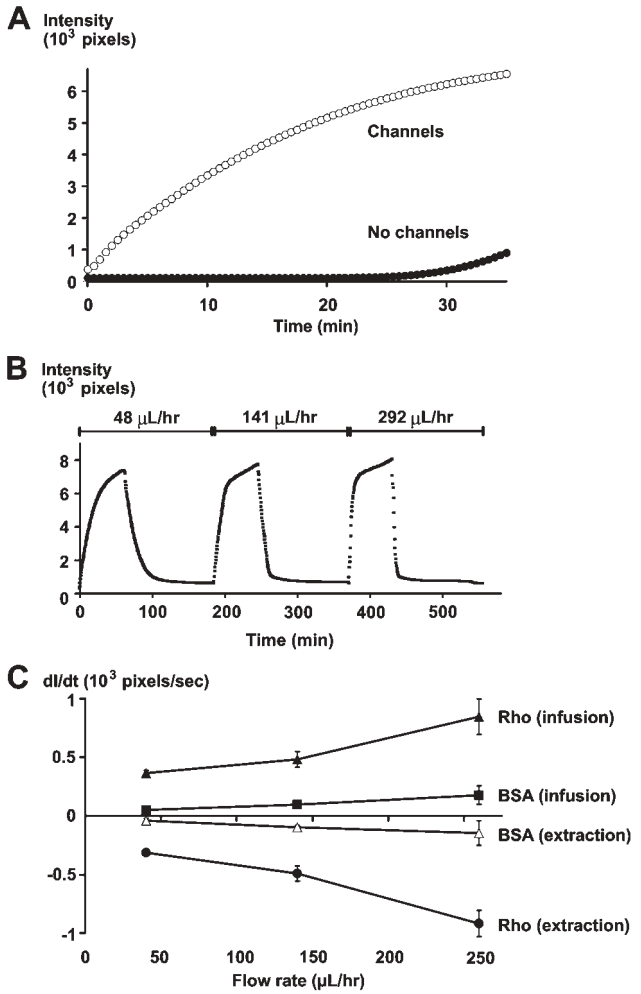

Fig. 4 Transport of rhodamine and BSA in microfluidic gels. (A) Representative plots of fluorescence intensity *versus* time for a collagen gel with channels and one without channels. A solution of rhodamine was placed at a driving pressure difference of 8 mm H_2O across each gel. (B) Representative plot of fluorescence intensity *versus* time for a microfluidic collagen gel under cycles of infusion and extraction of rhodamine at three flow rates and pressure differences: $\sim$48 μL h^{-1} (8 mm H_2O), $\sim$141 μL h^{-1} (30 mm H_2O), and $\sim$292 μL h^{-1} (50 mm H_2O). Intensities in (A) and (B) are given in normalized units. (C) A plot of the rate of intensity change d*I*/d*t* as a function of flow rate for rhodamine (Rho) and fluorescent BSA.

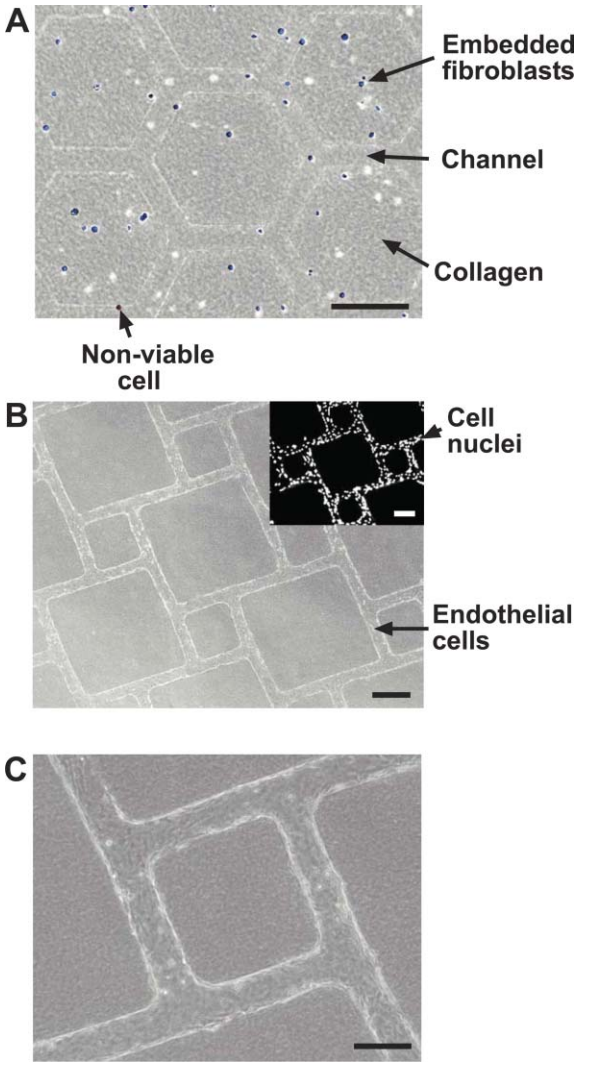

Fig. 5 Use of perfused microfluidic gels as scaffolds for cell culture. (A) Collagen gel with embedded fibroblasts. Nuclei of cells are stained with Hoechst 33342 (blue) and propidium iodide (red). Some nuclei appear unstained by Hoechst because they are too far from the plane of focus. (B) Collagen gel with a monolayer of endothelial cells lining internal channels. Inset, Hoechst-stained microvascular network. (C) Image of another microvascular network, at higher magnification. Scale bars refer to 200 μm.

within microfluidic collagen gels (Fig. 5A). Because these cells do not remain viable after extended times at room temperature, we modified our fabrication technique so that the gelation of collagen took place in two stages. First, fibroblast-containing liquid collagen precursor was applied around a gelatin mesh and gelled at room temperature for <15 min. We have found that this initial treatment rigidifies the precursor sufficiently to withstand melting of the mesh. Second, the materials were raised to 37 °C to melt the gelatin and to complete the gelation of collagen. This modified procedure yielded well-formed channels in gels that contained embedded cells. Co-perfusion with propidium iodide and Hoechst dye to label necrotic/late-phase apoptotic cells and all cells, respectively, showed that the vast majority (>96%) of embedded fibroblasts survived embedding and melting of gelatin. In particular, transient exposure to liquid gelatin did not appear to damage embedded fibroblasts, at least in the short term.

We also examined the viability of HDMECs seeded on the walls of collagen channels (Fig. 5B). When seeded as a dense suspension through open microfluidic networks in collagen gels, HDMECs attached, spread, and proliferated. After five days of culture, seeded cells remained localized to the internal surfaces of collagen networks, as shown by Hoechst stain. The cells deformed the initially sharp features of the networks, so that the corners became noticeably rounded after a few days in culture; extended perfusion of cell-free networks did not lead to deformation of features. Despite this cell-induced deformation, the networks remained open and sustained perfusion of culture media.

Conclusions

This work describes the formation of microfluidic networks in ECM gels by using molded gelatin as a sacrificial element. These gels allowed the transport of macromolecules into the gel under low driving pressure differences. Bulk gels also sustained transport, but required substantially higher pressure differences to achieve equivalent rates of delivery. We have shown that rhodamine and BSA readily diffused from channels into the gel, but 1 μm diameter microspheres did not. Rates of transport did not reach limiting values under the range of flow rates used.

Because the gels consisted of native ECM proteins, they served as natural scaffolds for cell culture. When seeded in channels, endothelial cells grew under perfusion to form a monolayer that lined the channels. Trace amounts of residual gelatin did not appear to grossly alter the survival, spreading, or proliferation of cells cultured on or in the gel.

This gelatin-based method of forming microfluidic gels has several advantages: First, it is subtractive and thus applicable to a variety of materials. In theory, our technique may be used to form channels in any hydrogel that polymerizes under conditions that do not melt gelatin (*i.e.* under $\sim$28 °C), and should be applicable to photopolymerized gels as well. Second, it uses a sacrificial material (gelatin) that is flexible, yet strong. Meshes made of concentrated gelatin resist deformation and fracture, thus allowing their manipulation. The denseness of the gelatin may inhibit diffusion of liquid precursor into the gelatin and thereby preserve the patency of channels at least as narrow as 6 μm; we have yet to identify the lower size scale at which this method can no longer form open channels. Third, it results in the formation of monolithic structures. These open gels can easily withstand substantial lumenal pressures in excess of those typically used in tissue engineering and in microfluidic devices.[25]

This procedure also has disadvantages: First, because the gelatin meshes are so flexible, they usually do not lie perfectly planar within the gel. While this distortion may not be critical for forming microfluidic networks, it may result in channels that are not completely identical. Second, because gelatin swells upon release, the channels that form are consistently wider than the original features in PDMS are. We expect that this limitation can be overcome by simply scaling all features so that they swell to the desired size. Third, this method is currently limited to planar networks and stacks of planar networks. It lacks the three-dimensional versatility of photolithographic methods, such as stereolithography, that form channels voxel-by-voxel.[12,26]

We expect that these microfluidic gels will serve as structures for the study of transport and for constant perfusion and growth of cultured cells. The use of gels that present desired functional groups for promoting cell adhesion or differentiation[27,28] should enhance the versatility of these materials.

Acknowledgements

This work was supported by the National Institute of Biomedical Imaging and Bioengineering (award EB005792, EB003157, and EB002228) and the Whitaker Foundation (RG-02-0344). This work made use of facilities at the Photonics Center at Boston University. We thank V. Falanga, G. Price, E. Damiano, and M. Tang-Schomer for useful discussions, and BASF for providing Pluronic F-127.

References

1 N. A. Peppas, Y. Huang, M. Torres-Lugo, J. H. Ward and J. Zhang, *Annu. Rev. Biomed. Eng.*, 2000, **2**, 9–29.
2 J. L. Drury and D. J. Mooney, *Biomaterials*, 2003, **24**, 4337–4351.
3 J. R. Levick, *Q. J. Exp. Physiol.*, 1987, **72**, 409–437.
4 K. Y. Lee, M. C. Peters, K. W. Anderson and D. J. Mooney, *Nature*, 2000, **408**, 998–1000.
5 S. Ramanujan, A. Pluen, T. D. McKee, E. B. Brown, Y. Boucher and R. K. Jain, *Biophys. J.*, 2002, **83**, 1650–1660.
6 M. Radisic, L. Yang, J. Boublik, R. J. Cohen, R. Langer, L. E. Freed and G. Vunjak-Novakovic, *Am. J. Physiol.: Heart Circ. Physiol.*, 2004, **286**, H507–H516.
7 C. P. Ng, C. L. Helm and M. A. Swartz, *Microvasc. Res.*, 2004, **68**, 258–264.
8 C. M. Nelson and J. Tien, *Curr. Opin. Biotechnol.*, 2006, **17**, 518–523.
9 K. M. Chrobak, D. R. Potter and J. Tien, *Microvasc. Res.*, 2006, **71**, 185–196.
10 M. Cabodi, N. W. Choi, J. P. Gleghorn, C. S. Lee, L. J. Bonassar and A. D. Stroock, *J. Am. Chem. Soc.*, 2005, **127**, 13788–13789.
11 R. B. Vernon, M. D. Gooden, S. L. Lara and T. N. Wight, *Biomaterials*, 2005, **26**, 1109–1117.
12 K. Arcaute, B. K. Mann and R. B. Wicker, *Ann. Biomed. Eng.*, 2006, **34**, 1429–1441.
13 S. Kaihara, J. Borenstein, R. Koka, S. Lalan, E. R. Ochoa, M. Ravens, H. Pien, B. Cunningham and J. P. Vacanti, *Tissue Eng.*, 2000, **6**, 105–117.
14 M. D. Tang, A. P. Golden and J. Tien, *Adv. Mater.*, 2004, **16**, 1345–1348.
15 G. M. Whitesides, E. Ostuni, S. Takayama, X. Jiang and D. E. Ingber, *Annu. Rev. Biomed. Eng.*, 2001, **3**, 335–373.
16 E. Kim, Y. N. Xia and G. M. Whitesides, *Nature*, 1995, **376**, 581–584.
17 J. L. Tan, W. Liu, C. M. Nelson, S. Raghavan and C. S. Chen, *Tissue Eng.*, 2004, **10**, 865–872.
18 M. D. Tang, A. P. Golden and J. Tien, *J. Am. Chem. Soc.*, 2003, **125**, 12988–12989.
19 K. A. Holtham and N. B. Slepecky, *J. Histochem. Cytochem.*, 1995, **43**, 637–643.
20 P. Klosen, X. Maessen and P. van den Bosch de Aguilar, *J. Histochem. Cytochem.*, 1993, **41**, 455–463.
21 N. L. Jeon, I. S. Choi, B. Xu and G. M. Whitesides, *Adv. Mater.*, 1999, **11**, 946–950.
22 J. H. Northrop and M. Kunitz, *J. Gen. Physiol.*, 1926, **10**, 161–177.
23 J. H. Northrop and M. Kunitz, *J. Gen. Physiol.*, 1926, **8**, 317–337.
24 M. Fialkowski, C. J. Campbell, I. T. Bensemann and B. A. Grzybowski, *Langmuir*, 2004, **20**, 3513–3516.
25 M. Radisic, W. Deen, R. Langer and G. Vunjak-Novakovic, *Am. J. Physiol.: Heart Circ. Physiol.*, 2005, **288**, H1278–H1289.
26 K. T. Nguyen and J. L. West, *Biomaterials*, 2002, **23**, 4307–4314.
27 M. P. Lutolf and J. A. Hubbell, *Nat. Biotechnol.*, 2005, **23**, 47–55.
28 J. Kisiday, M. Jin, B. Kurz, H. Hung, C. Semino, S. Zhang and A. J. Grodzinsky, *Proc. Natl. Acad. Sci. U. S. A.*, 2002, **99**, 9996–10001.