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Partitioning microfluidic channels with hydrogel to construct tunable 3-D cellular microenvironments

Amy P. Wong [a,b,1], Raquel Perez-Castillejos [b,1], J. Christopher Love [c,*], George M. Whitesides [b,**]

[a] Harvard Biophysics Program, Harvard Medical School, Boston, MA 02115, United States
[b] Department of Chemistry and Chemical Biology, 12 Oxford Street, Harvard University, Cambridge, MA 02138, United States
[c] Department of Chemical Engineering, Massachusetts Institute of Technology, Cambridge, MA 02139, United States

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Abstract

Accurate modeling of the cellular microenvironment is important for improving studies of cell biology *in vitro*. Here, we demonstrate a flexible method for creating a cellular microenvironment *in vitro* that allows (i) controlled spatial distribution (patterning) of multiple types of cells within three-dimensional (3-D) matrices of a biologically derived, thermally curable hydrogel (Matrigel) and (ii) application of gradients of soluble factors, such as cytokines, across the hydrogel. The technique uses laminar flow to divide a microchannel into multiple subchannels separated by microslabs of hydrogel. It does not require the use of UV light or photoinitiators and is compatible with cell culture in the hydrogel. This technique makes it possible to design model systems to study cellular communication mediated by the diffusion of soluble factors within 3-D matrices. Such factors can originate either from secretions of neighboring cells patterned within the microchannel, or from an external source – e.g., a solution of growth factors injected into a subchannel. This method is particularly useful for studying cells such as those of the immune system, which are often weakly adherent and difficult to position precisely with standard systems for cell culture. We demonstrated this application by co-culturing two types of macrophage-like cells (BAC1.2F5 and LADMAC cell lines) within spatially separated regions of a slab of hydrogel. This pair of cell lines represents a simple model system for intercellular communication: the LADMAC cells produce colony-stimulating factor 1 (CSF-1), which is required by the BAC cells for survival.

Keywords: Cellular signaling; Co-culture; Hydrogel; Microenvironment modeling; Micropatterning; Macrophage

1. Introduction

In vivo cellular behavior is complex: cells integrate and respond to numerous signals that originate both from their local microenvironment and from distant sources. These signals come from direct contact with contiguous cells (such as gap junctions in myocytes), from soluble factors secreted by neighboring or distant cells (such as growth factors and hormones), and from the surrounding extracellular matrix (ECM), which contributes both chemical and mechanical signals [1–3]. Intercellular communication is necessary for coordinating collective responses among multiple cells. One example is the type of inflammatory response in the immune system in which macrophages, recruited to a site of injury, secrete TNF (tumor necrosis factor) to activate leukocytes [4]. A second example occurs during wound healing, where fibroblasts and epidermal cells secrete IGF (insulin-like growth factor) to induce the formation of granulation tissue and the reepithelialization of the wound [5].

Several experimental methods are useful in studying cellular communication within three-dimensional (3-D) matrices. In transmigration assays, cells cultured in an upper chamber

* Corresponding author. Tel.: +1 617 324 2300; fax: +1 617 258 5042.
** Corresponding author. Tel.: +1 617 495 9430; fax: +1 617 495 9587.
E-mail addresses: clove@mit.edu (J. Christopher Love), gwhitesides@gmwgroup.harvard.edu (G.M. Whitesides).
[1] Contributed equally.

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coated with hydrogel respond to a gradient of cytokine that diffuses from a lower chamber through pores in the base of the upper chamber [6]. The bases of the two chambers are typically several millimeters apart. Cells suspended in uncured hydrogel can also be plated on Petri dishes, and – after gelation – exposed to a solution of cytokine that covers the layer of gel [7–9]. Suspending cells in liquid hydrogel followed by curing makes it possible to co-culture different types of cells.

The effectiveness of these methods to deconstruct the dependence of cellular behavior on the concentration of specific soluble biomolecules is limited, because they are not able to (i) control the position of and distance between cells on a length scale similar to that observed in tissue (i.e., a few to hundreds of microns) or (ii) expose the cells in culture to steady gradients of soluble factors that are not disturbed by convective flow [10].

Several techniques [11] have demonstrated that microfabrication makes it possible to reduce the dimensions of the structures of hydrogel. PDMS stamps fabricated by soft lithography can mold layers of most types of hydrogel, including biologically derived ones that gel thermally, but they can generate only planar patterns – or layered stacks of them [12–14]. Photolithographic techniques (those that use photochemical initiators, followed by exposure to UV light, to define the regions of cross-linked hydrogel) can produce more complex structures than those based on molding, but are limited to synthetic hydrogels that cure by UV light [15–17], and are often toxic to cells. Other methods to pattern hydrogels containing embedded cells rely on partially filling microchannels with gel (either the bottom half of a channel [18] or specific volumes within the channel delimited by arrays of micropillars [19] or by lateral walls [20]), and using those parts not filled with gel to perfuse the cells in culture. These approaches improve the versatility of forming microstructures of hydrogel for cell culture, but either simply replicate, at the microscale, analogous 2-D macroscopic substrates coated with gel, or require a new design of the microchannel for each new configuration of the structures of hydrogel.

This paper describes a method for studying intercellular communication between cells cultured within biologically derived, 3-D matrices of microscopic size. We use laminar flow to partition microchannels with one or more microslabs of hydrogel (Fig. 1a) into distinct, gel-separated subchannels. M. S. Kim et al. [21] used a similar technique to form slabs of a synthetic gel – i.e., Puramatrix, a self-assembling peptide hydrogel – in microchannels. The patterning process relies on thermal curing of the hydrogel – from a liquid state at 4 °C to a gelled state at 25–37 °C – and allows a single design of the microchannel to yield multiple experimental configurations. We demonstrate that this method makes it possible to (i) culture cells either within the slabs of hydrogel or on the surface of the subchannels created by the slabs, (ii) pattern different types of cells in adjacent structures of hydrogel, and (iii) apply gradients of soluble factors across the slabs of hydrogel containing cells.

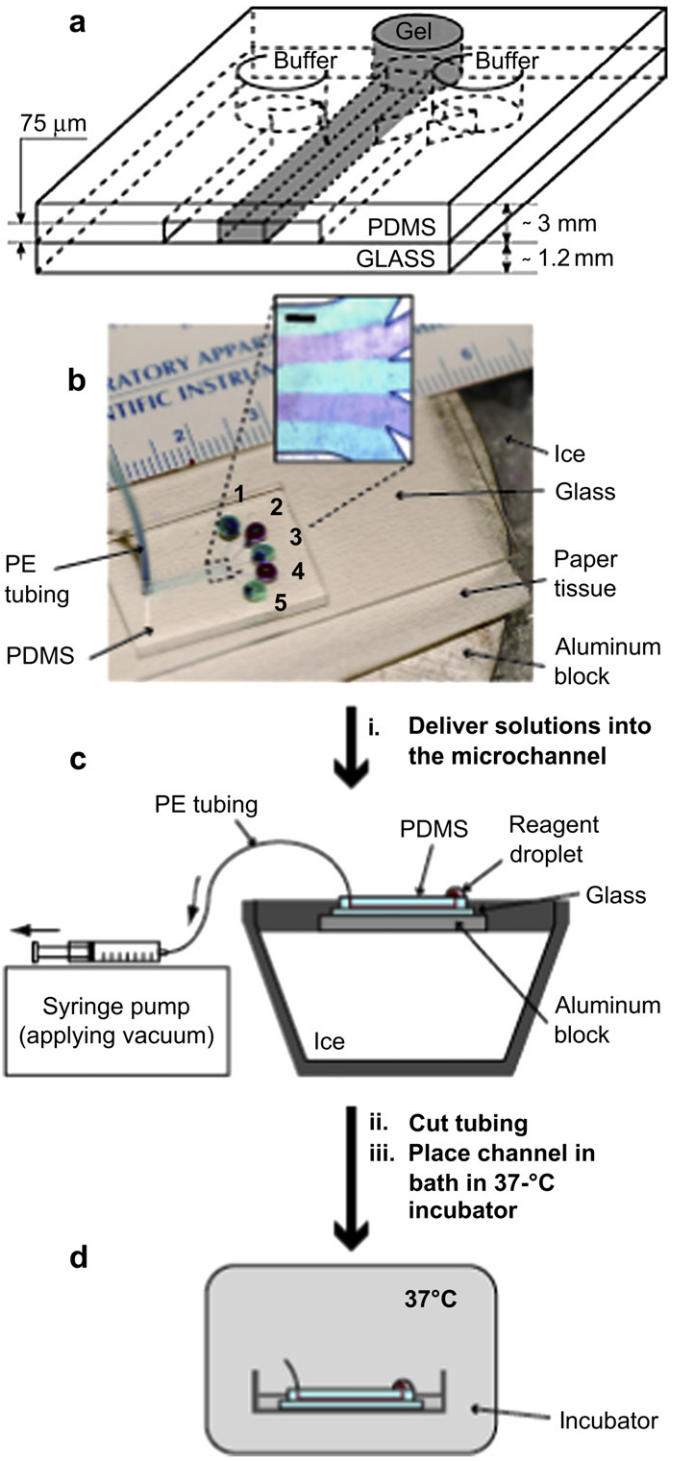

Fig. 1. Partitioning microchannels with slabs of hydrogel. (a) Schematic of a slab of hydrogel formed in the center of a microchannel by injecting simultaneously hydrogel in its liquid form (central stream) and buffer having similar viscosities (left and right streams). (b) Five-inlet channel on an aluminum plate resting upon ice; paper tissue was placed on the aluminum disc for easy visualization of the channel. Droplets of either hydrogel in liquid form or spacing solution (containing a blue dye here) were added to the inlets and delivered into the channel by applying vacuum with a syringe pump to the outlet. The inset shows an enlarged view of the laminar flow developed in the microchannel; (top left) scale bar: 300 μm. (c) Schematic (side view) of the experimental setup. (d) After injecting the spacing solution and liquid Matrigel, we stopped the flow suddenly by cutting the tubing at the outlet with scissors and immediately placed the channel in a sterile bath at 37 °C for 10 min.

2. Materials and methods

2.1. Cell culture

NIH/3T3 fibroblasts (ATCC; CRL-1658) were cultured at 10% CO_2 in Dulbecco's Modified Eagle's Medium (DMEM) supplemented with 4 mM L-glutamine, 4.5 g/l glucose, and 10% fetal bovine serum. Monocyte-derived LADMAC cells (ATCC; CRL-2420) were cultured at 5% CO_2 in Eagle's Minimum Essential Medium (MEM) with 1.5 g/l sodium bicarbonate and 10% fetal bovine serum. BAC1.2F5 macrophages (generously donated by Dr. E.R. Stanley; Albert Einstein College of Medicine, New York) were cultured at 5% CO_2 in alpha MEM (Eagle's Minimum Essential Medium, Alpha modification) supplemented with 10% newborn calf serum and 36 ng/ml recombinant human CSF-1. BAC cells were not cultured for more than 20 passages. We routinely co-cultured BAC and LADMAC cells in alpha MEM supplemented with 10% newborn calf serum and 36 ng/ml recombinant human CSF-1. The starving medium (used in experiments where LADMAC cells were the only source of CSF-1) did not contain any recombinant CSF-1. As a control, prior to co-culture, we grew LADMAC cells in starving medium for a week and confirmed that the proliferation of LADMAC cells was not affected by the change of medium. We also grew BAC cells in medium where LADMAC cells had been cultured for two days, and verified that the presence of LADMAC-secreted CSF-1 instead of recombinant human CSF-1 did not modify the proliferation and survival of BAC cells.

2.2. Fabrication of PDMS-on-glass microchannels

We coated a 3-inch silicon wafer with a thin (~75 μm) layer of photoresist and patterned it by photolithography to form the master [22]. We poured PDMS onto the silanized master and allowed it to cure overnight at 60 °C. We punched holes through the replicas of PDMS for the inlets and outlets of the microchannels, and sealed the replicas irreversibly to glass slides by exposing both pieces to an air plasma and placing the treated surfaces in contact [23]. The microchannels were then sterilized by rinsing them with 70% ethanol, brought into a cell-culture hood, and rinsed thoroughly with sterile PBS. Detailed methods regarding the fabrication of the microchannels by soft lithography are available (Supplementary information, section S2).

2.3. Formation of slabs of hydrogel in microchannels

During the formation of the slabs, we kept all required tools (microchannels and pipette tips) and solutions (Matrigel, spacing solution, and suspensions of cells) at ~4 °C; the microchannels rested on a flat, aluminum disc placed on ice (Fig. 1b). We used polyethylene tubing to connect the outlet of the channel to a gas-tight syringe that was operated by a syringe pump. We kept the polyethylene tubing filled with 200 mg/ml PEG-8000 solution to maintain a uniform pressure drop throughout the fluidic system consisting of the tubing and the microchannel. All the elements of the experimental setup (except the syringe pump) were sterilized with 70% ethanol and brought into the cell-culture hood. After rinsing the channel thoroughly with PBS, we deposited droplets (6 μl) of the appropriate solutions (buffer or liquid Matrigel – pure or containing cells) on each inlet. After depositing the droplets on the inlets, we started the syringe pump to suction the droplets into the channel at a flow rate of 15 μl/min. As soon as the channel was filled with the solutions, we cut the tubing at the outlet with scissors to stop the flow, and immediately placed the channel in a sterile bath at 37 °C for 10 min. We then rinsed out the spacing solutions with PBS, deposited additional drops of PBS on every inlet and outlet to prevent the hydrogels from drying out, and used the microchannels within two days.

2.4. Formation of slabs of hydrogel containing viable cells

We prepared a concentrated suspension of cells in chilled culturing medium, and mixed it with liquid Matrigel at a ratio of 80:20 in volume (MG:cell solution). We then deposited 6-μl droplets of either the suspension of cells in liquid Matrigel, or of the buffer on each inlet, and delivered them into the microchannel by applying vacuum at the outlet with a syringe pump.

2.5. Fluorescent staining of cells

To assess the viability of cells, we stained them with a solution of propidium iodide (PI; 0.668 kDa) at 100 μg/ml in the culturing medium required by each type of cell. We added ~20 μl of PI solution to the inlets of the microchannels, allowed PI to diffuse into the slabs of gel for 15 min, rinsed the channels with PBS, and imaged the samples. To stain the membrane of cells for confocal imaging, we incubated the cells in a solution of Vybrant DiI (5 μl of the stock solution in 1 ml of culturing medium) for 15 min.

2.6. Microscopy

We imaged the channels using a charge-coupled device camera (CCD camera) on an inverted, epifluorescence microscope and processed the images with MetaMorph and Adobe Photoshop. We false-colored the fluorescence of Figs. 2 and 5 and increased their contrast using Photoshop.

2.7. Image analysis

To obtain the profiles of concentration of TRITC-dextran as it diffused through the slabs of gel, we took images (16-bit TIFF images) of the samples at several timepoints and converted them to 8-bit JPEG (511 × 639 pixels) images with Adobe Photoshop. With MATLAB, we chose 10 randomly distributed, horizontal lines per JPEG image and extracted the light intensity of each pixel. We sum-averaged the intensity of each pixel for the 10 lines so that one intensity profile characterized each image. We then subtracted the background and normalized the intensity profile of each image by dividing the intensity of each pixel by the maximum intensity of the image. The diffusion constants were obtained from correlating the normalized intensity profiles and the equation describing diffusion through the slab of gel (Supplementary information, section S8).

3. Results and discussion

3.1. Formation of microslabs of hydrogel in microchannels

The technique described here takes advantage of steady, spatially defined flows of fluid within the laminar regime [24] to form slabs of thermally cured hydrogel inside microchannels. Under conditions of laminar flow (that is, Reynolds numbers less than ~2000; see Supplementary information, section S1, for more details on the physical principles of laminar flow), fluids tend to flow parallel to the walls of the microchannel, there is no turbulence, and mixing between streams occurs only by diffusion (Supplementary Fig. 1) [25]. We hypothesized that delivering a stream of hydrogel (in its liquid form) flanked with two streams of buffer, followed by rapid thermal gelation, would make it possible to form self-standing structures of hydrogel inside the microchannel, provided that the diffusion of hydrogel into buffer and vice versa was small (Fig. 1a). Since the purpose of these buffer streams is to define the structure of hydrogel spatially, we name them ''spacing solutions''. After gelation, the spacing solutions can be rinsed out and replaced by other solutions (e.g., culturing media or solutions of cytokines).

Microchannels were fabricated by soft lithography using standard protocols to generate replicas of a mold in

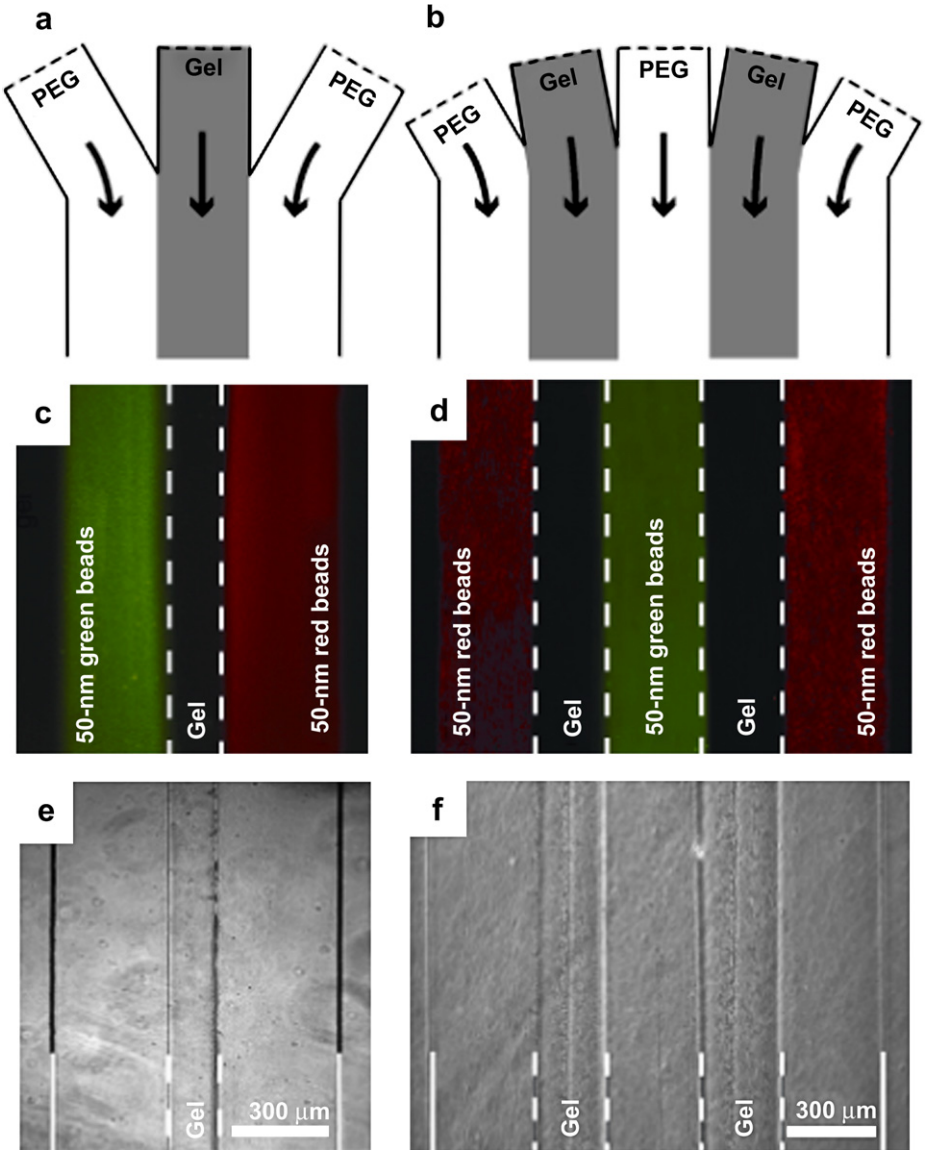

Fig. 2. Formation of individually addressable subchannels inside a microchannel. Schematic of the distribution of flowing solutions (hydrogel in liquid form and PEG spacing solution) to form one (a) or two (b) wall-like structures of gel in microchannels having three (a) and five inlets (b), respectively. (c and d) After curing, the microslabs of gel divided the microchannel into subchannels that were accessible through the corresponding inlets. We added suspensions of fluorescent 50-nm Nile Red polystyrene beads and 50-nm Yellow Green polystyrene beads to the subchannels. The beads remained in their respective compartments for more than 30 min. (e and f) Phase-contrast images corresponding to the fluorescent images of panels c and d, respectively.

poly(dimethylsiloxane) (PDMS) [23]. After sealing the PDMS replicas to glass slides irreversibly, we sterilized the PDMS-on-glass microchannels by rinsing them with 70% ethanol, brought them into a cell-culture hood, and rinsed them thoroughly with sterile phosphate-buffered saline (PBS). From this step on, the channels were kept under sterile conditions.

For the experiments described in this paper, we designed three different microchannels – with three, four, or five inlets. All had a single outlet (Supplementary Fig. 2a–c). The width of the inlet channels (w_i) was 300 μm for all designs, and the width of the main channel (W), where all the inlet channels converged, was $W = n \times w_i = n \times 300$ μm, where n is the number of inlets of each design ($n = 3$, 4, or 5); the length of the main channel was ~10 mm and the height of the channels was ~75 μm (Supplementary Fig. 2d). The Reynolds numbers for an aqueous solution flowing at 15 μl/min in these microchannels were less than one in all cases (between 0.17 for the five-inlet device and 0.28 for the three-inlet device) and the resulting flows were laminar, as expected [25].

Among the large number of hydrogels proposed in the literature for cell culture [7,8,26–28], we chose to use the Growth Factor Reduced Matrigel matrix (MG) for four reasons: (i) it is biologically derived – it is a soluble extract of the basement membrane of murine tumoral epithelia, mainly composed of laminin and collagen IV; (ii) it is commercially available, and has been used extensively both *in vivo* and *in vitro* in the culture of many types of cells; [29] (iii) it is liquid at 4 °C, but gels within 15 min at 22–37 °C, and (iv) it is sufficiently stiff after gelling [9] to

form self-standing structures of gel. The main disadvantage of Matrigel is that because of its biological origin, it contains not only structural proteins, but also growth factors and enzymes. To minimize the variability introduced by these additional growth factors, we chose Matrigel in its purified form, i.e., Growth Factor Reduced Matrigel.

As the spacing solution in these experiments, we used a solution of PEG (220 mg/ml polyethylene glycol 8000 in PBS) that had the same viscosity as liquid MG (15 mPa s). This polymer is nontoxic and biocompatible; [28] the viability of NIH/3T3 fibroblasts cultured in the presence of 220-mg/ml PEG-8000 for more than seven days was not affected. Solutions with a viscosity of 15 mPa s flowing at 15 μl/min along the channels described in Supplementary Fig. 2, yielded Reynolds numbers within the range of laminar flows: between 0.01 (five-inlet channels) and 0.02 (three-inlet channels). Matching the viscosity of the solutions minimized the dependence of the width of the streams flowing in microchannels upon the differences in viscosity between the fluids forming each stream (Supplementary Fig. 3).

We formed microslabs of hydrogel by delivering liquid MG and spacing solution into the microchannels under conditions of laminar flow at 4 °C, and caused MG to gel *in situ* by raising the temperature to 37 °C. We started by placing a microchannel — filled with PBS — on a chilled, aluminum disc (Fig. 1b and c) and connected its outlet to a syringe pump. Immediately after depositing a droplet (6 μl) of liquid MG or of spacing solution to each of the inlets, we applied vacuum to the channel by starting the syringe pump at a withdrawing flow rate of 15 μl/min (Fig. 1c). As soon as the channel was filled with MG and PEG solutions, we cut the tubing at the outlet with scissors to immediately stop the flow, transferred the channel to a sterile, 37 °C bath, and allowed the slabs to gel for 10 min (Fig. 1d). The number of inlets of the channel and the distribution of solutions to the inlets determined the final arrangement of structures of hydrogel inside the channel. In Fig. 1b, for instance, if droplets of gel were added to inlets B and D, and spacing solution to A, C, and E, two wall-like structures of hydrogel would form after curing, separated from each other and from the walls of the channel.

After gelation, the inlets for delivering liquid MG became plugged and could no longer pass fluid. The inlets used for delivering spacing solutions, however, remained open and could be used afterwards to access the parts of the channel not filled with gel — that is, the subchannels (Fig. 2). The width of the gelled microslabs was 315 ± 21 μm — as it results of measuring the widths of 10 different slabs formed in microchannels having 300-μm-wide inlet channels. We determined that the microslabs of hydrogel extended from top to bottom of the channels by filling each of the subchannels with a suspension of green- or of red-fluorescently labeled 50-nm beads (Fig. 2c and d). After allowing the system to equilibrate for ~30 min (no additional solutions were delivered after filling the subchannels) we observed that each type of bead remained in its respective subchannel without crossover.

3.2. Cells cultured next to or within microslabs of Matrigel inside microchannels remain viable

The technique allows the culture of cells either next to the slabs of hydrogel or within them. To examine the viability of cells grown on the surface of the subchannels, we partitioned a microchannel with one slab of hydrogel (as described above), rinsed out the spacing solution with PBS, and then filled the subchannels with a solution of fibronectin (FN) in PBS (50 μg/ml). After incubating the channel for 1 h at 37 °C and 10% CO_2, we rinsed the subchannels with fresh PBS, filled them with a concentrated suspension of NIH/3T3 fibroblasts (~10^6 cells/ml) in culturing medium, and allowed

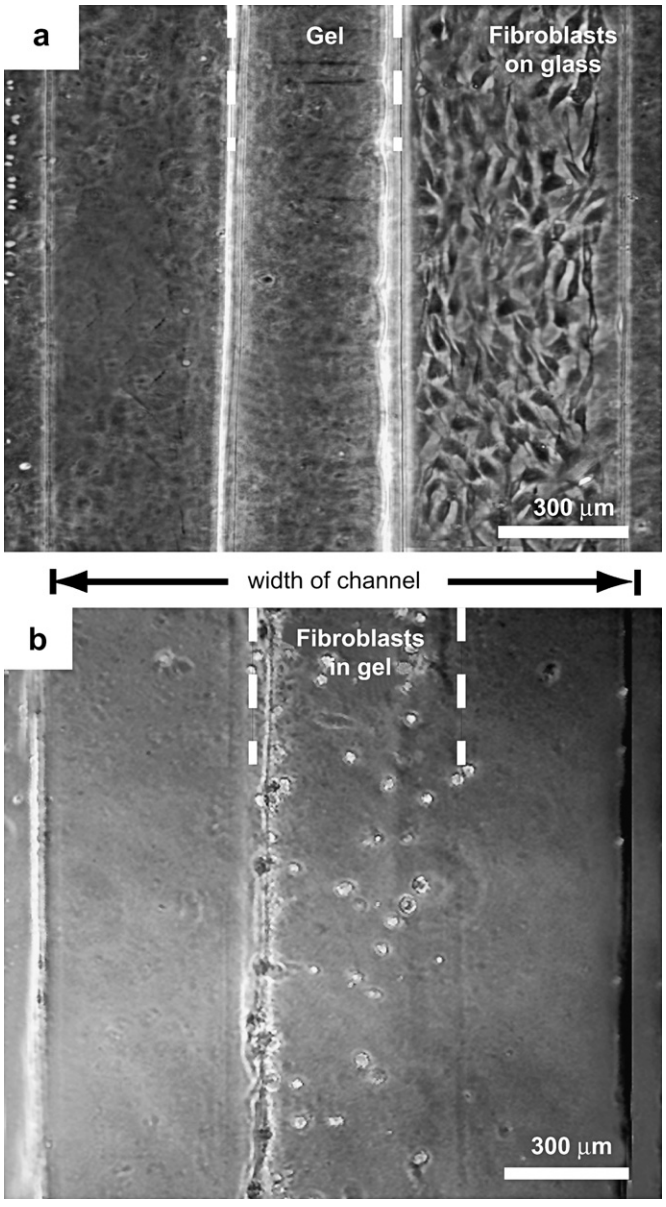

Fig. 3. Culture of cells next to or within a slab of hydrogel. (a) Phase-contrast image of NIH/3T3 fibroblasts cultured on the surface of a subchannel adjacent (right) to a slab of hydrogel. (b) Phase-contrast image of NIH/3T3 fibroblasts after two days in culture within a slab of hydrogel (center) in a microchannel. The microfluidic systems in (a) and (b) were different.

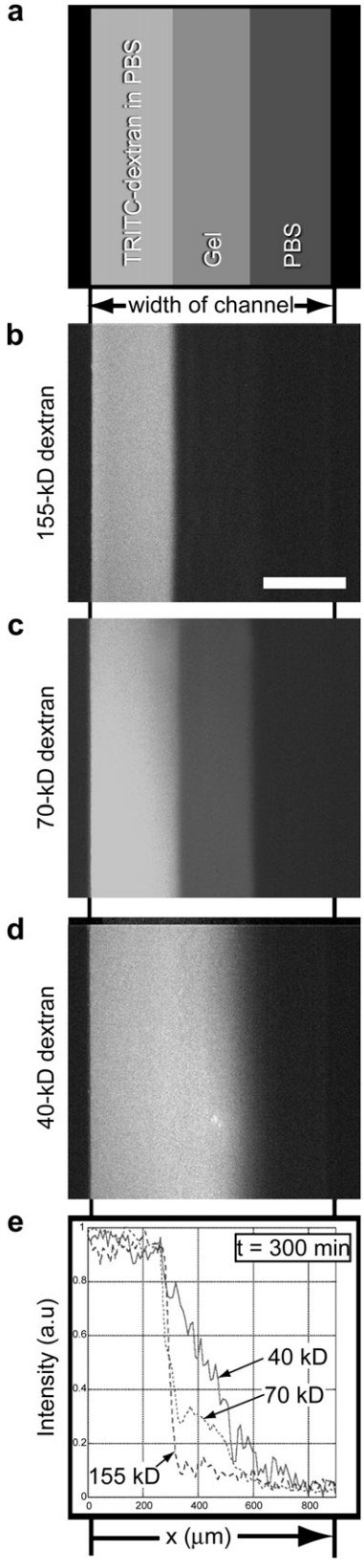

Fig. 4. Generation of gradients of molecules across a slab of hydrogel. (a) Schematic (top view) of a three-inlet channel with a slab of hydrogel at the center and two adjacent subchannels. A solution of TRITC-labeled dextran (40 kDa, 70 kDa, or 155 kDa) at 0.5 mg/ml in PBS was injected (~5 μl/min) into the left subchannel, and PBS was injected into the right subchannel (~5 μl/min). (b–d) Fluorescent images of the microchannel after injecting the solutions into the subchannels for 300 min. (e) The fluorescence intensity profiles reached by diffusion of TRITC-dextran with different molecular weights (after 300 min) were plotted against the width of the channel.

the cells to settle by gravity in the 10% CO_2 incubator. Within ~4 h, the cells attached to the FN-coated glass floor of the subchannel adjacent to the slab of hydrogel (Fig. 3a) and showed the same spread morphology as those cultured on Petri dishes. We cultured fibroblasts in these subchannels for up to a week (renewing the culturing medium every 1–2 days). The cells remained alive and proliferated to confluence on the floor of the subchannel.

We also cultured murine fibroblasts within microslabs of MG. We studied the effect of shear on the cells in liquid MG. In pressure-driven flows, velocity is not constant over the cross section of the channel, resulting in shear stress on the cells as they flow. Shear stress on cells (5–15 dyn/cm^2; 10 μN = 1 dyn/cm^2) occurs *in vivo* and is necessary for endothelial cells to develop properly [30]. Excessive shear stress, however, can damage the cellular membrane and compromise the viability of the cells [31]. Shear stress can be decreased by lowering the flow rate. Experimentally we observed that decreasing the flow rate at which solutions were delivered, resulted in microslabs that did not extend from top to bottom of the channel. To form microslabs we used the flow rate that allowed the cells to remain viable and allowed the microslabs to extend from top to bottom of the channel (Supplementary Table 1).

We deposited 6-μl droplets of either a suspension of cells in liquid MG (20% v/v; ~10^5 cells/ml) or of spacing solution, on each inlet and delivered them into the microchannel by applying vacuum to the outlet with a syringe pump at flow rates between 5 μl/min and 110 μl/min. To assess the viability of the cells embedded in the gelled slabs, a solution of propidium iodide (PI) in culturing medium (100 μg/ml) was added to the subchannels and incubated for 30 min. We chose PI to characterize our technique because it reports the effect of shear stress on the integrity of the plasma membrane of cells. We also injected suspensions of nanometer-sized beads to the subchannels (as described in Fig. 2) to determine if the structures of hydrogel extended the entire height of the channel. We determined that flow rates between 10 μl/min and 20 μl/min yielded slabs of hydrogel that extended from top to bottom of the channel and maximized the viability of the embedded cells (>90%).

The difference in morphology of NIH/3T3 fibroblasts cultured on a 2-D surface (the base of the subchannel) and in a 3-D matrix (within a slab of MG) is obvious by comparison of Fig. 3a and b. As reported in the literature [9,29], fibroblasts present well-defined lamellipodia when they are attached to stiff substrates, while their morphology in compliant (not loaded mechanically) gel is rounded. A series of images taken with a confocal microscope of a single volume of gel shows how fibroblasts that adhered to the substrate formed lamellipodia, but those cells fully embedded in gel adopted the spherical shape typically observed *in vivo* (Supplementary Movie 1). Cells cultured within the slab of hydrogel remained viable (>90% cells alive) for at least one week; we renewed the culturing media every 1–2 days.

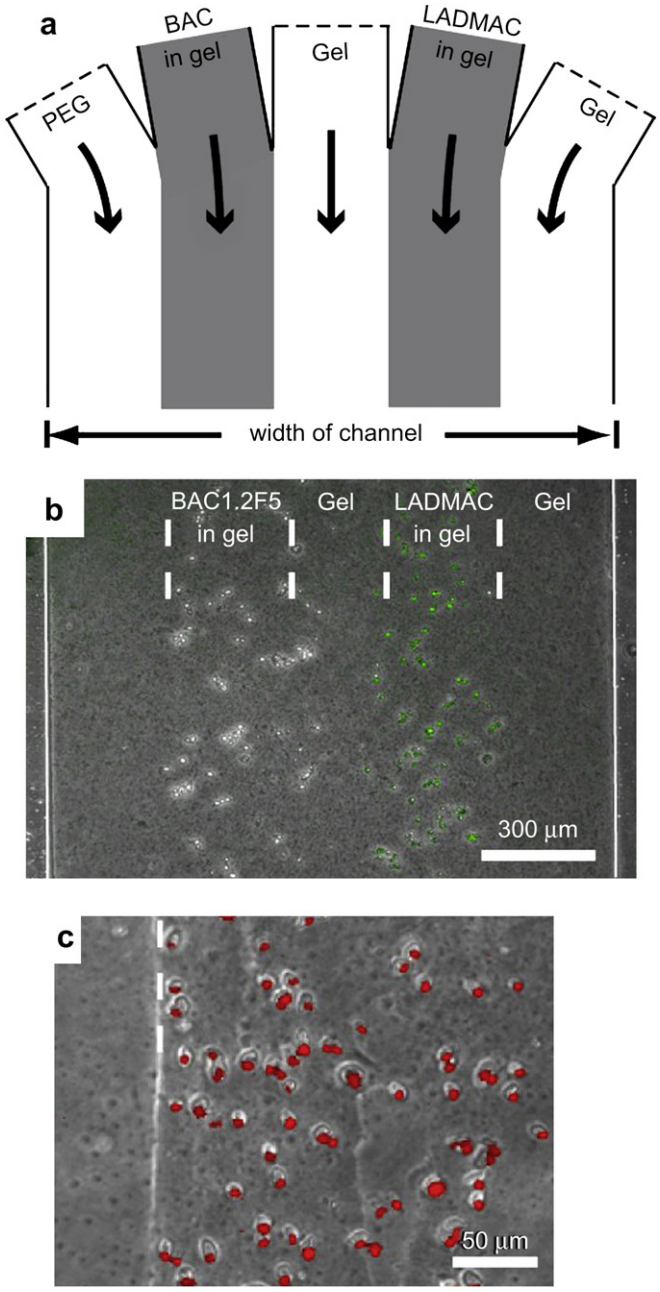

Fig. 5. Intercellular communication between BAC and LADMAC cells cultured within a slab of hydrogel. (a) Schematic (top view) of the solutions injected into a five-inlet channel to form a system of adjacent slabs of hydrogel: (from left to right) spacing solution, BAC cells suspended in liquid Matrigel, pure gel, LADMAC cells in gel, and pure gel. (b) Slabs of hydrogel formed inside of a microchannel according to the distribution of solutions described in panel a. (c) BAC cells stained with propidium iodide (false-colored in red) after being cultured for two days without any source of CSF-1 – either from culture medium or from LADMAC cells.

3.3. Transport of solutes by diffusion across the structures of hydrogel

Since convective flow is prevented through gels, we reasoned that steady gradients of soluble biomolecules could be generated across a wall-like structure of hydrogel by delivering a solution containing the biomolecules to one of the adjacent subchannels and allowing it to diffuse. We designed experiments to investigate the diffusion of soluble biomolecules through microslabs of hydrogel (Fig. 4a). After gelation, we replaced the spacing solution in one of the adjacent subchannels with a 0.5-mg/ml solution of fluorescently-labeled dextran (TRITC-dextran) in PBS (left in Fig. 4a) and that in the other subchannel with PBS (right in Fig. 4a). The molecular weights of cytokines and dyes commonly used in cell biology are within the range of those of the TRITC-dextran molecules used in our experiments: 40 kDa, 70 kDa, and 155 kDa. To maintain a constant concentration of dextran in each subchannel, we maintained a continuous flow of the solutions in the subchannels (~5 μl/min) during the experiments. As expected, the experiments showed that transport by diffusion occurs more rapidly for TRITC-dextran with a lower molecular weight than for one with a higher molecular weight (Fig. 4). After 5 h, the concentration of 40-kDa TRITC-dextran decreased linearly across the gel (Fig. 4d and e). The concentration of 155-kDa TRITC-dextran, on the contrary, was almost zero within the slab of hydrogel (Fig. 4b and e). The calculated values of the diffusion constant for 40-kDa, 70-kDa, and 155-kDa TRITC-dextran through slabs of Matrigel are 0.45 $\mu m^2/s$, 0.34 $\mu m^2/s$, and 0.18 $\mu m^2/s$, respectively. These data suggest that it should be possible to use the slabs of hydrogel as semi-permeable barriers to exclude embedded cells from biomolecules of particular sizes (e.g., 155-kDa antibodies) while permitting their exposure to other small cytokines (e.g., insulin, TGF-β).

3.4. Communication of co-cultured BAC and LADMAC cells through diffusion of CSF-1

In vitro studies of the communication between cells would benefit from techniques that allow spatial positioning of different types of cells within a 3-D matrix in such a manner that cellular communication occurs by means of secreted soluble biomolecules. We chose two murine cell lines – adherent, macrophage-like BAC1.2F5 cells [32] (BAC cells) and weakly adherent, monocyte-derived LADMAC cells [33–35] as a simple system for testing intercellular communication. For survival, BAC cells require the presence of CSF-1 [32], a growth factor secreted by LADMAC cells [33] that stimulates the survival and proliferation of macrophages. To the best of our knowledge, the co-culture of BAC and LADMAC cells has not been reported.

Inside of a five-inlet microchannel, we formed a ensemble consisting of one subchannel (far left in Fig. 5a and b) and four adjacent slabs of hydrogel: (i) BAC cells embedded in gel (center left in Fig. 5a and b), (ii) a slab of pure MG (center in Fig. 5a and b), (iii) a slab of gel containing LADMAC cells (center right in Fig. 5a and b), and another slab of pure MG (far right in Fig. 5a and b). Immediately after gelation, we replaced the spacing solution in the subchannel with culturing medium (i.e., alpha MEM supplemented with 10% newborn calf serum and 36 ng/ml of recombinant human CSF-1). After 12 h, we injected culturing medium lacking

recombinant CSF-1 (starving medium) into the subchannel; under these conditions, the only source of CSF-1 available to the BAC cells was the nearby LADMAC cells. We renewed the starving medium in the channels daily, and in the presence of LADMAC cells, BAC cells remained viable for a week in the channel. To confirm that the presence of LADMAC cells was necessary for the proliferation of BAC cells in starving medium, we formed an ensemble consisting of the same adjacent slabs of hydrogel as described above (Fig. 5a and b), but lacking the LADMAC cells. In these devices, BAC cells died within two days after the addition of starving media (Fig. 5c).

4. Conclusions

This paper describes a method to generate tunable systems that model cellular microenvironments *in vitro*. This method is based on constructing microfluidic systems that consist of a PDMS channel partitioned by slabs of biologically derived Matrigel. This technique to form gel-partitioned microfluidic systems presents three advantages when compared to other techniques presently available for the culture of cells in 3-D matrices *in vitro*: (i) it does not make use of any element potentially toxic to cells (ultraviolet light, photoinitiators); (ii) it can be used with biologically derived hydrogels under conditions that are compatible with cell culture (temperatures of ~4 °C up to ~37 °C); and (iii) it allows many configurations of slabs of gel using a single design of the microchannel. The slabs of hydrogel provide a 3-D matrix for cell culture that can be spatially defined with a range of dimensions (heights of 50–100 μm and widths of 200–500 μm) similar to the length scales relevant for cells organized in tissues [10]. We have shown that uneven distributions of biomolecules can be produced in gel-partitioned microfluidic systems by two methods: (i) by injecting the appropriate solutions (buffer with and without a chosen biomolecule) into the subchannels (Fig. 4), or (ii) by patterning various types of cells that secrete different cytokines, within separated, but adjacent, microslabs of hydrogel (intercellular communication between BAC and LADMAC cells in Fig. 5). Gel-partitioned microfluidic systems enable patterning cells (even weakly adherent cells such as LADMAC cells) within 3-D matrices, and tuning their environment through controlled addition/removal of soluble factors. These characteristics make this technique a powerful and versatile tool for modeling of *in vivo* cellular microenvironments. We propose that it will be useful and suitable for studying intercellular communication mediated by soluble factors.

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Appendix. Supplementary information

Supplementary information associated with this article can be found in the online version, at doi:10.1016/j.biomaterials.2007.12.044.

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