



ANNUAL
REVIEWS **Further**

Click [here](#) for quick links to Annual Reviews content online, including:

- Other articles in this volume
- Top cited articles
- Top downloaded articles
- Our comprehensive search

Cell Culture Models in Microfluidic Systems

Ivar Meyvantsson¹ and David J. Beebe²

¹Bellbrook Labs, LLC, Madison, Wisconsin 53711;
email: ivar.meyvantsson@bellbrooklabs.com

²Department of Biomedical Engineering, University of Wisconsin at Madison,
Madison, Wisconsin 53706; email: djbeebe@wisc.edu

Annu. Rev. Anal. Chem. 2008. 1:423–49

First published online as a Review in Advance on
March 4, 2008

The *Annual Review of Analytical Chemistry* is online
at anchem.annualreviews.org

This article's doi:
10.1146/annurev.anchem.1.031207.113042

Copyright © 2008 by Annual Reviews.
All rights reserved

1936-1327/08/0719-0423\$20.00

Key Words

microenvironment, extracellular matrix, microfabrication

Abstract

Microfluidic technology holds great promise for the creation of advanced cell culture models. In this review, we discuss the characterization of cell culture in microfluidic systems, describe important biochemical and physical features of the cell microenvironment, and review studies of microfluidic cell manipulation in the context of these features. Finally, we consider the integration of analytical elements, ways to achieve high throughput, and the design constraints imposed by cell biology applications.

1. INTRODUCTION

We continuously strive toward a better understanding of human biology and disease. Because direct observation of humans is only possible in epidemiological studies, experimental work must rely on biological models. Models are, to varying degrees, able to manipulate and analyze biological systems. They can be categorized in order of biological relevance as follows: (*a*) biochemical models using purified biomolecules, (*b*) cell lines, (*c*) cultured primary cells, (*d*) model organisms such as yeast and fruit flies expressing endogenous or human genes, (*e*) human tissue explants, and (*f*) animals (often rodents or nonhuman primates). However, increasing biological relevance goes hand in hand with increases in cost, labor, and experiment duration. Consequently, the simplest model that sufficiently represents the system of interest is usually chosen so that the largest possible parameter space can be explored using available resources.

Mammalian cell culture can in many cases provide both the desired biological relevance and throughput, and it represents a large fraction of human biology research. The year 2007 marked the one-hundredth anniversary of *in vitro* cell culture. Although our understanding of molecular and cell biology has increased tremendously over the past 100 years, the methods used today are surprisingly similar to those employed by Harrison in his 1907 study of frog neurons cultured in hanging drops of clotted frog lymph on a depression slide (1). We still rely on undefined biological material (e.g., fetal bovine serum) and simple containers such as dishes, bottles, and flasks. Currently, studies of model organisms and animal models are more biologically relevant than those using cultured human cells, but this may change in the future with the introduction of improved cell culture systems.

Various influences determine the phenotype of cells *in vivo*, including interactions with neighboring cells, interactions with the extracellular matrix (ECM), and systemic factors. Ease of use and low price notwithstanding, dishes and flasks allow no control over the spatial distribution of the cells and biomolecules needed to model many chemical and physical influences cells experience *in vivo*. Approaches that increase the biological relevance of cell culture models while maintaining or increasing the throughput of current methods are of great interest to the life sciences community.

Microfluidic systems represent a new kind of cell culture vessel that expands our ability to control the local cellular microenvironment (2, 3). Microfluidic systems enable patterning of molecules and cells (4) as well as both passive (5) and active (6) cell handling and environmental control. Temporal and spatial control on the micrometer scale (0.1–100 μm) have been used in fundamental studies from the subcellular (7) to the organismal (8) level, for instance in studies of cell division axis orientation (9) and geometric influence on cell survival (10).

Applications of soft lithography in cell biology have been reviewed (11), as have methods of engineering cellular interactions via microtechnology (4). Microfabricated cell cultures were reviewed by Voldman et al. (2), Park and Shuler (12), and more recently by El-Ali et al. (3). This review explicitly focuses upon cell biology and the manner in which microfluidic structures have been or may be employed

to manipulate the cellular microenvironment in order to better understand cell biology or to build cellular models and assays. We also discuss analytical methods used with microfluidic cell culture, ways in which throughput can be increased, and the constraints imposed upon microfluidic system design by cell biology applications.

2. MICROFLUIDIC CELL CULTURE

The cell culture methods in use today have been founded on over a century of work. It is important to put microfluidic cell culture in context with this work to determine which assumptions hold true and which do not when methods are scaled down to microchannels. Although this characterization is just beginning, several research groups have already contributed to a better understanding of the multiple aspects of microfluidic environments.

The physical design of microfluidic devices affects the cell microenvironment of cultured cells. Design considerations for useful application of microfluidic devices in cell biology were described by Walker et al. (13), who introduced the concept of effective culture volume as an indicator of cellular control over the microenvironment in the culture device. The complex but predictable patterns formed by growth factors and other solutes have been described as secondary interfaces (14), the shapes of which are affected by perfusion of the cell culture medium. A comprehensive review of perfusion culture system design, material choices, and operation was recently presented by Kim et al. (15), whose discussion of both the engineering aspects and biological application considerations of these factors provided a practical overview of design, fabrication, sterilization, culture, and analysis. In this section, we describe relevant microfluidic cell culture work to date both in two dimensions (i.e., suspension and monolayer) and in three dimensions (i.e., cells in a polymer matrix).

2.1. Cell Culture in Suspension and Monolayers

Fluid suspension is the natural environment of several cell types, including yeast cells and mammalian blood cells. Other cell types retain important phenotypic characteristics in monolayer culture. This is true for cell-cell attachments that are generally conserved in monolayer culture of epithelial cells. Suspension cell culture is traditionally performed in roller bottles or spinner flasks, whereas monolayer cell culture is done in Petri dishes, multiwell plates, or culture flasks.

One of the first studies of adherent cell culture in microfluidic channels was performed by Tilles et al. (16). They constructed a microchannel cell culture system from polycarbonate and glass with channels 85–500 μm in height and a cell culture area 25 mm wide \times 75 mm long. Primary rat hepatocytes were seeded in coculture with 3T3-J2 fibroblasts in channels that had either a gas exchange membrane top or a polycarbonate top. A significant difference in cell viability and hepatocyte function was seen after only 8 h when the devices with and without gas exchange membrane were compared. Monitoring albumin and urea production (markers of hepatocyte

function) in devices with a gas exchange membrane over time for different flow rates, the authors found that increasing flow rate led to diminished viability and function. At low flow rates, hepatocyte function remained stable for 10 d.

As the liver is a highly perfused organ, perfusion culture is an appropriate way to model the mass transport aspects of liver biology *in vitro*. The culture of a human hepatocarcinoma cell line (Hep G2) in a perfusion culture device was reported by Leclerc et al. (17). They built a network of 270- μm -high channels constructed from two layers of poly(dimethylsiloxane) (PDMS). Leclerc et al. used 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid (HEPES)-buffered media (i.e., independent of carbon dioxide) and reported successful attachment, spreading, and growth on the PDMS surface; they also reported that gas transport through PDMS was sufficient to maintain a viable culture over days. They further compared perfusion and static conditions. Glucose consumption was similar in both cases for 3 d, after which the cells in static culture started to die. In perfusion culture, the cells continued to grow and consume glucose at an increasing rate until confluence was reached around day 7. The authors also monitored albumin expression, which is an indicator of normal phenotype of the Hep G2 cell line. Albumin production was comparable for the first 3–4 d in perfusion and static culture, after which it dropped sharply in static culture, consistent with the observed cell death. Although nutrient requirements and rate of waste production were shown to vary widely for different cell types, this study demonstrates that there is a time window wherein these needs are met in static culture.

Yu et al. studied the effects of microchannel dimensions on the proliferation of suspended insect cells (Sf9) (18) as well as the effects of cell density, exogenous growth factors, and media change frequency on the growth rate of normal murine mammary gland cells (NMuMG) (19). They found that the growth rate of Sf9 cells decreased with increasing cell density and decreasing channel height, whereas channel width and length did not affect cell proliferation. Interestingly, the authors showed that NMuMG cells proliferated more rapidly in microchannels than in 96-well plates, all else being equal. The difference between microfluidic channels and wells was decreased when the cell culture medium was changed frequently (either every 1 h or every 4 h). The frequent media change also reduced the growth advantage provided by fetal bovine serum supplementation over epidermal growth factor (EGF) supplementation only. Consistent with the effective culture volume concept discussed above (13), the authors hypothesized that the secreted growth factor accumulation facilitated by the diffusion-dominated environment of microfluidic channels was responsible for this effect.

Other indications of the environmental differences between microfluidic systems and conventional culture come from embryo studies. Raty et al. cultured murine embryos in microfluidic devices and observed a greater proliferation rate with daily media changes in the microfluidic device compared to conventional methods (20).

2.2. Three-Dimensional Cell Culture

In vivo tissue organization and three-dimensional cell culture play an important role in cells' behavior. Three-dimensional cell culture can increase the biological relevance of

cell-based models beyond that achievable in monolayer culture. Dolberg's and Bissell's study of Rous sarcoma virus (RSV) infection is a striking example of the powerful influence exerted by complex three-dimensional environments *in vivo* (21). RSV is known to cause neoplastic transformation of chick tissues and cells derived from chick embryos, but when Dolberg and Bissell infected cells of the early chick embryo, the activity of the highly potent oncogene possessed by the virus was completely inhibited, even in the fully grown organism. When removed from the animal the cells expressed a transformed phenotype after only 24 h in culture, demonstrating that the ability of *in vivo* three-dimensional architecture to control structure and function is lost when the cells are removed from the animal. These aspects of cell behavior can only be modeled in three-dimensional culture (22).

Several approaches to seeding cells in gels inside microchannels have been reported. Toh et al. produced a device with a middle channel for cell seeding in a gel flanked on each side by aqueous channels for perfusion. The perfusion channels were separated from the culture channel by rows of micropillars (23). The authors cultured several cell types in the device and, over several days, analyzed cell functions including albumin secretion of primary hepatocytes and differentiation competence of mesenchymal stem cells. Laminar flow was used to pattern a microchannel with a hydrophobic coating, which led to the formation of virtual walls along the channel (J.P. Puccinelli & D.J. Beebe, manuscript in preparation). Subsequently, laminar flow was again used to pattern two separate cell types in collagen gel along the hydrophilic center line of the channel, leaving the hydrophobic edges of the channel unwetted. The unwetted area was subsequently filled with culture media, thereby providing an aqueous interface to the cell culture.

Kim et al. created another device that employed laminar flow to create perfusion channels on either side of a gel compartment (24). They cultured hepatocellular carcinoma cells in the device and compared their albumin secretion when cultured in collagen, PuraMatrixTM (a synthetic peptide provisional matrix), or polylactic acid (PLA). They found that albumin secretion was initially lower in PuraMatrixTM than either collagen or PLA, but became higher by day three and remained high throughout the eight-day duration of the study. Cells cultured in collagen and PLA showed similar rates of albumin secretion.

Paguirigan and Beebe molded channels from gelatin by crosslinking with the enzyme transglutaminase (25). This enzyme occurs naturally; thus, the device contained no synthetic crosslinking or photoinitiating agents. The authors reported that the morphology of cells grown on the gelatin surface of these channels was significantly different from that of cells in monolayer culture on tissue culture-treated polystyrene. The former showed nuclear staining results, indicating cell entry into the three-dimensional crosslinked collagen matrix.

3. CELL MICROENVIRONMENT

The cell microenvironment comprises a complex of biochemical and physical influences (which may be synergistic or antagonistic), the frequency (26) and time sequence (27) of which can be remembered by cells. However, the cell

microenvironment and the part it plays in homeostasis are still poorly understood (28). With regard to the soil nematode *Caenorhabditis elegans*, for example, every single cell division leading to the formation of the hatched larvae has been mapped (29), the entire genome of the organism has been sequenced (29), and numerous published reports have described various details of *C. elegans* cell biology. Still, we cannot take a cell or tissue from the nematode and maintain its natural phenotype in culture because the biochemical and physical influences necessary to reproduce the cell microenvironment that maintains that phenotype remain unknown. Microfluidics provides a set of tools that may enable us to define specific features of in vitro cell culture environments.

3.1. Biochemical Environment

The healing of wounded skin illustrates several biochemical aspects of cell microenvironments including gradients, soluble factor signaling, gas transport, and interactions with the ECM (**Figure 1**). When a blood vessel breaks, a cascade of events leads to the formation of a clot that plugs the vessel to prevent blood loss. Immediately, platelets

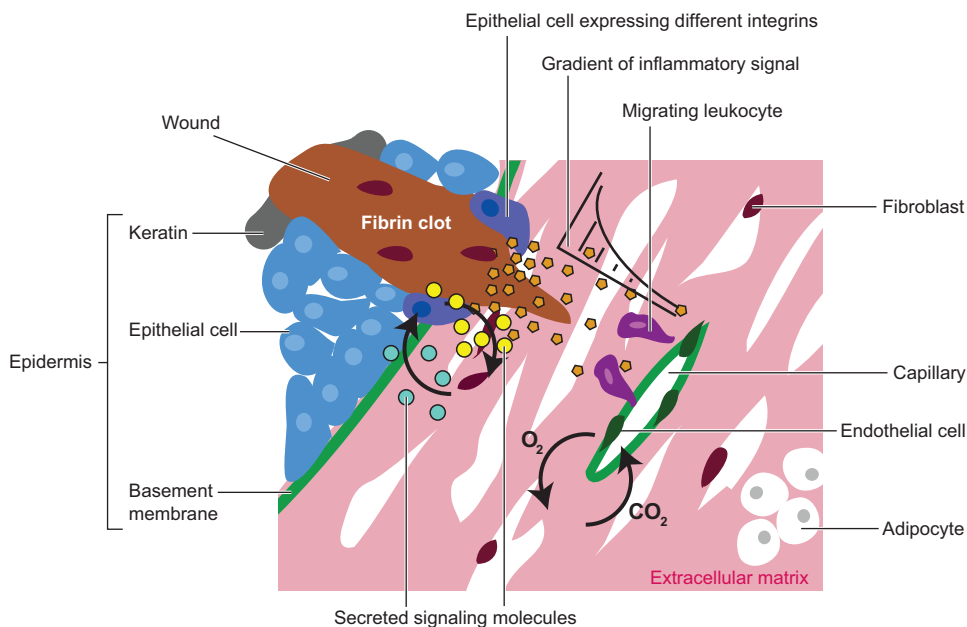


Figure 1

The biochemical environment of wound healing. A fibrin clot has formed to plug the severed blood vessel. Macrophages and platelets in the clot release cytokines that form a gradient and mediate recruitment of leukocytes from the blood stream via chemotaxis. As the epithelium regenerates the skin in the wounded area, epithelial cells interact with the extracellular matrix via integrin receptors and with stromal cells (e.g., fibroblasts and adipocytes) via soluble factors.

and resident macrophages begin to release soluble factors, known as cytokines, that mediate inflammation. As cytokine molecules diffuse away from the wound, they form a gradient. When the gradient reaches the closest intact blood vessel, the cytokine signal is sensed by local endothelial cells and soon by leukocytes in the blood stream. This signal prompts endothelial cells to mediate leukocyte entry into tissue and leukocytes to enter the tissue (30). Leukocytes move up the cytokine gradient, a behavior known as chemotaxis.

Later in the healing process, skin epithelial cells proceed to regenerate the epidermis (31). The cells of the dermal connective tissue, including fibroblasts and adipocytes (collectively known as stromal cells), regulate epithelial proliferation and differentiation via soluble factors (32). Integrin receptors allow epithelial cells to sense and bind to the ECM and are specific for a given set of ECM proteins. In normal skin, epithelial cells lie on the basement membrane, a layer of ECM rich in laminin. During regeneration, the epithelial cells express new integrin receptors in order to interact with the fibrin clot and the dermis and re-epithelialize the wounded area (31). Another important biochemical influence is gas concentration: As the wound continues to heal, new blood vessels are formed to provide gas exchange to the regenerated tissue. Oxygen plays important roles in metabolism, in the regulation of angiogenesis (discussed in detail below in the context of physical environmental influences), and in the zonal differentiation in the liver (33). These biochemical aspects of the cell microenvironment and the ways in which they have been explored with microfluidic systems are described in this section.

3.1.1. Soluble factors. Various biomolecules exist dissolved intracellularly and in the interstitial space. Many actions in the body are regulated, at least partially, by soluble factors including angiogenesis (34) and embryonic morphogenesis (35). A molecule secreted from a cell is transported away from the source via diffusion, the convective flow of the blood stream, and the continuous flow from capillaries into lymphatic vessels. The spatial and temporal distribution of soluble factors is further affected by the lifetime of the factor dictated by the molecule's inherent stability or enzymatic degradation, sequestration by the ECM, and binding endocytosis by other cells. Microfluidic systems have been employed to perform spatially defined treatment. For instance, Sawano et al. used laminar streams to expose only a small part of a cell to EGF. They subsequently observed the intracellular propagation of cell signaling (7). Similarly, Blake et al. demonstrated the use of laminar flow for treatment of specific regions of perfused rat brain slices (36). They measured electrical activity related to respiratory motor function and showed that this activity could be suppressed in a specific area of the slice by applying the appropriate solution.

Spatially defined treatment is often impossible to achieve with conventional means. Microfluidic systems provide new opportunities in achieving spatially defined treatment by facilitating readout (see, e.g., Reference 7) and in studies of spatial heterogeneity of responses and spatially defined signals (see, e.g., Reference 36).

3.1.2. Gradients. Many soluble factor signals, in particular those associated with chemotaxis, exist as gradients in vivo. Using microfluidic networks with two

inlets, Jeon et al. generated gradients via successive flow splitting and diffusive mixing (37). Under continuous flow the concentration distribution can be maintained stably over time. The authors studied the migration of primary neutrophils in gradients of interleukin-8 (IL-8) and observed the differential sensitivity of neutrophils to a sharp versus gradual spatial drop in concentration. Wang et al. demonstrated that although MDA-MB-231 cells did not show a chemotactic response to linear gradients of EGF, they did show significant directional migration when exposed to polynomial gradients (38).

In another exploration of the temporal aspects of neutrophil migration, Irimia et al. employed a gradient-switching device to study the response of neutrophils to time-dependent step-up, step-down, and reversal of IL-8 gradients (39). Interestingly, the authors observed a period of depolarization followed by repolarization when stepping down the gradient to half of the initial concentration, thereby revealing dynamics that had not been reported before. Employing an extremely low flow rate (314 nL/min), Mao et al. quantified the migration of *Escherichia coli* bacteria (40). In contrast to previous work with mammalian cells, the *E. coli* bacteria were suspended during the assay and were collected in different channels downstream depending on their position within the laminar flow. In the absence of gradients the bacteria were distributed symmetrically around the center channel. The authors successfully characterized the responses of wild-type and chemotactic mutant strains to several gradients, yielding results consistent with the known behavior of the strains. Flow-based gradients have also been used to explore the concentration dependence of proliferation and differentiation of human neural stem cells (41).

All of the above-mentioned approaches to gradient generation rely on continuous flow to maintain the gradient. Walker et al. found that although total migration distance was not influenced by flow rate, the direction of migration was biased in the direction of flow in a flow rate-dependent manner (42). An additional concern for flow-based gradients is the dissipation of endogenous signaling molecules. Cells generally do not act alone, but rather orchestrate their concerted action through soluble factor signals. Under flow conditions, even relatively slow flow, these signaling molecules are transported away from the cells that would otherwise respond (43). Thus, flow-free gradient devices facilitate the inclusion of cell-cell signaling in microfluidic cell culture models.

Several methods have been reported to produce gradients in microfluidic devices without flow. Abhyankar et al. characterized a device employing a 0.2- μ m pore-sized membrane to limit flow in a narrow channel between a large volume source and a sink (44). Using a hydrogel sandwiched between two layers of PDMS, one of which contained a channel network, Wu et al. demonstrated the formation of a gradient (45). The gradient varied linearly in one dimension across the hydrogel and different concentration profiles were achieved along the length of the microchannels by producing channels that traced a variety of curves across the plane of the hydrogel.

Generation of three-dimensional gradients in gels has also been shown. Rosoff et al. demonstrated the formation of arbitrary gradients via micropump printing on the top face of a collagen gel (46). Using three parallel channels molded in an agarose

gel, Cheng et al. formed a gradient in the center channel by using the two outermost channels as chemoattractant source and sink (47). They studied the behavior of *E. coli* as well as HL-60 cells exposed to appropriate gradient stimuli.

A variety of gradient generation tools have been reported, providing researchers with a choice of several approaches, including continuous-flow devices for fast switching and no-flow devices for inclusion of cell-cell signaling. Microfluidic gradient devices are a great improvement over pipette-based methods, Dunn and Boyden chambers, and transwell plates in terms of precision and variety of gradient shapes. However, the ease of use and throughput of microfluidic gradient devices can still be improved.

3.1.3. The extracellular matrix. The coupling of secreted factor distribution with flow in gel culture is considered in a recent review by Griffith and Swartz (48). Aided by computational models, the authors demonstrate the complex patterns formed by convective transport of secreted factors that interact with the ECM to enzymatically release secondary sequestered factors. These are some of the many functions of ECM in cell biology. As mentioned above, cell attachments to the ECM are not merely mechanical anchors, but receptors that sense the biochemistry and mechanics of the microenvironment. The use of synthetic biomaterials containing bioactive ligands to guide tissue morphogenesis in vitro was recently reviewed (49).

A surface-bound gradient of the ECM protein laminin was produced using a flow-based gradient (50) in the manner described above [see, e.g., Jeon et al. (37)]. Rat hippocampal neurons were grown on this gradient and the orientation of axon growth was observed. The orientation was found to be in the direction of increasing laminin concentration. Also, Tan and Desai produced multilayer cocultures with the goal of incorporating the cellular heterogeneity of blood vessels (51). They seeded each cell type in a gel and relied on the inherent contraction of the gel to form a channel on top of the gel for the next layer. Frisk et al. also took advantage of gel shrinkage to form an aqueous channel for perfusion of three-dimensional culture and relied on an array of micropillars to hold the gel in place (52). They also demonstrated rapid switching between perfusion solutions. The duration from the point at which 10% relative concentration of the new solution was reached to the point at which 90% relative concentration was reached (the rise time) was only 2 min. The authors further demonstrated viability of fetal monkey kidney (COS 7) cells after 72 h in culture. Evans et al. employed micropatterned three-dimensional ECM to study the direction of spiral ganglion neurite outgrowth by laminin and fibronectin (53). They observed outgrowth patterns consistent with the hypothesized role of ECM patterns in neurite guidance.

Tsang et al. produced three-dimensional hepatic tissues by photopatterning of poly(ethylene glycol) (PEG) hydrogels containing cells (54). The researchers incorporated cell-adhesive peptides, representing specific ECM proteins, in the hydrogels to support hepatocyte survival. From the same research group, Underhill et al. studied the function of liver cells embedded in PEG hydrogels (55) and found that albumin secretion of mouse embryonic liver cells was significantly influenced by the peptide sequence incorporated in the PEG hydrogel.

As described above, several methods have been reported to seed cells in three-dimensional gels. The results discussed in this section clearly show the exciting opportunities that exist for combining microfluidic patterning of biological matrices and rapid solution-switching with three-dimensional cell culture. These methods are important in the effort to establish appropriate spatial and temporal patterns of biochemical influences.

3.1.4. Gas concentration. Mammalian cell metabolism depends upon a regulated oxygen supply and the removal of carbon dioxide. To perform these processes in microfluidic cell culture systems, we must design the systems such that supply and use are well balanced. Supply depends on the device geometry, material, and perfusion conditions, whereas use depends on cell type and cell density. Oxygen concentration in microfluidic bioreactors has been measured during cell culture by means of an oxygen-sensitive ruthenium dye (56) and was found to decrease with increasing cell density.

A perfusion bioreactor system was demonstrated to form steady-state oxygen gradients in cell culture (57). The bioreactor was fabricated by machining from polycarbonate, which is relatively impermeable to oxygen. The reactor was perfused with an oxygenated cell culture medium and the flow rate was adjusted to achieve the desired gradient between the input and output due to cell consumption. It was shown that the pattern of oxygen tension-related enzyme expression (cytochrome P450 2B) in primary rat hepatocyte cultures is consistent with that shown *in vivo*. This model was further extended by adding fibroblasts in coculture with hepatocytes (58), and was recently applied to study the expression profile of hypoxic primary hepatocytes (59).

Multicompartment devices (cell culture analogs) have been constructed for toxicology studies (12). In some cases these devices have a gas-permeable area intended to model gas exchange in the lungs. An alternate way to regulate oxygen concentration was presented by Park et al., who employed water electrolysis to control the oxygen tension in a separate microfluidic chamber (60). Arbitrary spatial concentration profiles can be created by varying electrode geometry. Hyperoxic apoptosis of C2C12 myoblasts was also demonstrated.

A range of oxygen levels can be established in conventional cell culture, but this requires external gas regulation or culture media level adjustment. Microfluidic systems offer faster response times than external gas regulation; also, they do not depend upon cell culture media volume. Furthermore, perfusion of oxygenated media into gas-impermeable microfluidic devices with active culture has been shown to allow the formation of gradients that promote zonation in liver cell culture (57).

3.2. Physical Environment

Physical influences such as force and temperature can be sensed directly by cells, and other physical factors—including geometry—affect cells indirectly. Many physical influences exist in the vascular system. As discussed in the previous section, cells depend on constant transport and exchange of oxygen and carbon dioxide. When the geometry of the capillary network is structured such that transport in a certain

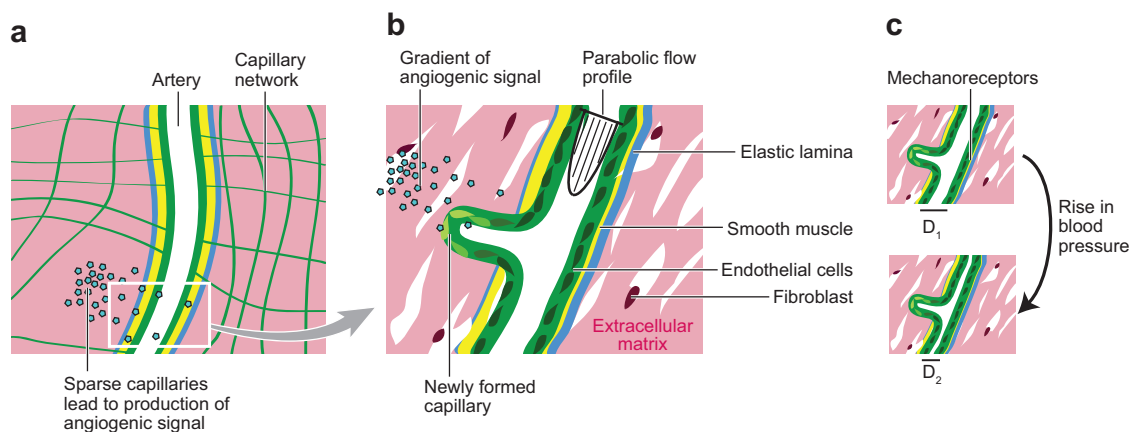


Figure 2

Physical environment of blood vessels. (a) An insufficiently oxygenated area of an artery and its associated capillary network. Oxygen-sensitive transcription factors cause the cells in that area to express angiogenic factors. (b) Local blood vessels respond to the angiogenic factors by expanding the capillary network in a “sprouting” fashion. The flow velocity profile in the artery is shown, as is the construction of arterial walls with endothelial cells lining the vessels and with an external covering of smooth muscle and elastic lamina. (c) The smooth muscle cells have mechanoreceptors that contribute locally to the regulation of blood pressure. When blood pressure rises, the receptors allow the smooth muscle cells to sense the change in vessel wall tension and respond by contracting the vessel to bring flow back to the previous level.

area is insufficient, cells sense the condition via oxygen-sensitive transcription factors such as hypoxia-inducible factors (HIFs) (61). In areas that are insufficiently perfused, HIFs lead to the expression of factors that promote angiogenesis, such as vascular endothelial growth factor (**Figure 2a,b**).

The velocity profile of blood flow is shown in **Figure 2b**. Blood flow causes shear stress on the endothelial cells lining blood vessels as well as on the leukocytes attached to vessel walls (62). The smooth muscle cells that surround arteries and arterioles are responsible for regulating blood flow; when blood pressure changes suddenly, smooth muscle cells detect the change in vessel expansion via mechanoreceptors and act to maintain the same flow rate to the downstream capillary network (63) (**Figure 2c**). In this section we discuss the ability of microfluidic systems to control these physical parameters of the cell microenvironment.

3.2.1. Fluid flow. Fluid flow in cell biological systems leads to shear stresses as well as local transport and sometimes systemic distribution of soluble factors. A wide range of fluid velocities exist in the human body. The velocity in large blood vessels may measure up to 0.3 m/s (300,000 $\mu\text{m/s}$) in the aorta (64), but then slows down immensely as the vascular network expands over the entire volume of the body at the capillary level. Interstitial flow from the vascular system to the lymphatic system is typically in the range of 0.1–1 $\mu\text{m/s}$ (48).

Bone tissue responds to mechanical signals, including shear stress in culture. The activity of the enzyme alkaline phosphatase (ALP) is a marker of bone cell function. Leclerc et al. studied the ALP activity of mouse calvarial osteoblastic (MC3T3-E1) cells in a PDMS microfluidic network (65). The authors found that although high flow rates led to loss of activity and eventually cell death, lower flow rates increased ALP activity up to threefold after 13 d in culture compared to static culture with media changes each day.

Using an array of 12 microfluidic bioreactors, Figallo et al. explored the differentiation of human embryonic stem cells to endothelial cells as determined by expression of α -smooth muscle actin (66). By comparing two different culture chamber designs, the authors showed that cultures in chambers producing higher shear stress developed a greater fraction of differentiated cells. For both chamber designs, lower seeding density also resulted in a greater fraction of differentiated cells.

Schaff et al. constructed a device to monitor leukocyte interaction with a biological substrate under flow conditions (67). The authors validated the device using primary human neutrophils and mouse fibroblasts transfected with human E-selectin. The capture, rolling, and deceleration to arrest were monitored under flow conditions. The cells were found to exhibit the expected response to activation by IL-8.

Microfluidic systems provide a variety of methods to control flow rate and modulate shear stress. These methods have been used to model physical influences on cells including endothelial cells, leukocytes, and osteoblasts.

3.2.2. Tissue mechanics. The mechanical properties of the cell microenvironment are an important influence on cell behavior (68). The cell-surface molecules that are responsible for anchoring cells to two-dimensional substrates, three-dimensional matrices, and neighboring cells act as receptors as well as anchors. For example, each member of the integrin family binds a specific set of ECM proteins (69). Integrin receptor complexes are coupled to the cytoskeleton. Ingber has described the processes of mechanical and chemical signal integration on the whole-cell level in the context of tensegrity models (70, 71). Such models present a view of sensing on the whole-cell level rather than on the individual mechanoreceptor level. In three-dimensional cell culture, for instance, the density of the ECM has been shown to influence cell differentiation (72). Similarly, McBeath et al. found that cell shape as dictated by the adhesive area available to cells in two-dimensional culture influenced lineage commitment of human mesenchymal stem cells (73). The authors showed that commitment to adipose or osteoblastic lineages was signaled through the cytoskeletal regulation protein RhoA. Micropatterning has also been used to elucidate various aspects of cell mechanical and spatial sensing (9, 10, 74, 75).

3.2.3. Geometry. The formation of branched duct structures (branching morphogenesis) in the breast may be guided by a geometrical influence analogous to the vascular remodeling discussed above. Nelson et al. used three-dimensional micropatterning to define mammary epithelial cell colonies in a collagen gel (76) and found that the synthetic tubules branched out at positions determined by the geometry of

the tubule. The observed branching pattern was consistent with the pattern of an unknown diffusible inhibitor.

Embryoid bodies (EBs) are spherical aggregates of embryonic stem cells (ES cells) and are commonly formed as a first step in ES cell experiments. Torisawa et al. demonstrated synchronized formation of uniformly sized EBs using a microfluidic device (77). They showed that the size of EBs could be controlled by channel cross-sectional geometry. Similarly, Karp et al. employed PEG microwells to control EB shape (78).

In an effort to reproduce aspects of liver tissue structure, Powers et al. constructed three-dimensional culture scaffolds via deep reactive ion etching in silicon (79). They selectively deposited a cell-adhesive coating on the inside walls of the culture chambers, as the top surface of the device and the bottom surface of each chamber did not support cell attachment. Interestingly, the authors found that cells precultured as spheroids fared better in the devices than those seeded from a single-cell suspension. This suggests the importance of the ECM in three-dimensional culture: During spheroid formation cells will synthesize ECM, which then becomes an integral part of the spheroid. Similarly, Gottwald et al. produced polymer microcontainer arrays for three-dimensional cell culture, and analyzed gene expression of hepatocellular carcinoma cells cultured in the device (80). They identified several genes that were upregulated in cells cultured in the device rather than in cells in conventional monolayer culture.

Using differently sized patches of clot-inducing tissue factor, Kastrup et al. studied the threshold response of blood clotting (81). They compared the threshold responses of platelet-rich and platelet-poor plasma as well as the importance of specific coagulation cascade factors.

Recently, Hui and Bhatia described a micromechanical device that allowed temporal control of cell-cell interaction (82). Their system was based on a structure made of microfabricated silicon combs, two of which could be separated and brought into close contact or brought into proximity without contact. Using this system, Hui and Bhatia analyzed the communication of hepatocytes and supporting stromal cells and found that a short period of contact followed by soluble factor communication is sufficient to maintain hepatocellular phenotype.

Tao et al. compared the fate of retinal progenitor cells (RPCs) cultured on porous and nonporous poly(methylmethacrylate) scaffolds after implantation into the sub-retinal space of mice (83). Although RPCs grew equally well on both substrates in vitro, cell survival, differentiation, and integration into host tissue in vivo was significantly greater for porous substrates than nonporous. Upon integration with the microenvironment provided by the mouse eye, the RPCs differentiated and expressed neuronal, glial, and retina-specific markers.

Precise microfluidic geometries have been utilized to model important influences in the body, ranging from mammary morphogenesis to blood-clotting threshold response. As evident from the studies discussed above, geometric effects are often the result of biochemical influences. Microfluidics enables the study of unknown biochemical influences [see, e.g., Nelson et al. (76)], as well as influences with complex kinetics [see, e.g., Kastrup et al. (81)].

3.2.4. Temperature. Cell behavior is influenced to a large extent by the temperature of the cells' environment. Given the small thermal mass and the large surface area:volume ratio of microfluidic systems, temperature is a particularly important consideration for microfluidic cell culture devices. Along with gene expression (84), diffusion and biochemical reaction kinetics change as a function of temperature. Microfluidic devices have been used to provide temperature-controlled environments for biological studies.

By placing a *Drosophila* embryo in a microfluidic device straddling the interface between two streams at different temperatures, Lucchetta et al. (8) observed the dynamics of embryonic patterning arising from the bicoid morphogen. The authors detected lower cell density and slower patterning in the cooler half of the embryo's body, followed by compensation that evened out the pattern across the entire body.

Differential flow control from two inputs at different temperatures was used by Pearce et al. to control the temperature in a cell culture chamber (85). The chamber had an electrode array on the bottom surface to monitor the electrical activity of neurons. The firing rates of dorsal root ganglion neurons were measured to demonstrate the operation of the system.

Microfluidic systems have proven useful for high-resolution control of temperature in biological studies. These would be difficult to do in conventional culture.

3.3. Compartmentalization

Two of the advantages of PDMS are its elasticity and its ability to conform to surfaces. These properties have been exploited for valving (86) and loading of bacteria into miniature culture chambers (87). For example, valves were employed by Balagadde et al. to construct small-volume bioreactors (88). The authors used the bioreactors to study quorum sensing and reported that population density via broadcasting and sensing of bacteria population was autonomously regulated, resulting in population oscillation. The small isolated volume of the reactor enabled monitoring at single-cell resolution. Compartmentalization has also been employed to specifically manipulate or analyze different parts of a single cell or individual cells that are biochemically connected to other cells via gap junctions.

Neural cell bodies and axons have distinct properties and functions. Taylor et al. employed a two-compartment microfluidic system to culture polarized neurons with cell bodies and axons in separate compartments (89). The authors studied axon-specific gene expression and regeneration after physical axotomy.

One symptom common to several diseases of the nervous system is distal-to-proximal axonal degeneration. Recently, Ravula et al. demonstrated the division of the environment surrounding a neuron into two distinct fluid compartments, one containing the cell body and the other containing the axon (90). Using the compartmentalized culture device, Ravula et al. settled a long-standing debate regarding the source of this degeneration and showed that a chemical insult to the axon, but not the cell body, led to the death of the axons.

In a similar experiment, Klauke et al. placed cardiomyocytes in microfluidic devices with two individually addressable compartments (91). The authors harvested

pairs of rabbit cardiomyocytes maintaining end-to-end connections, used micromanipulators to place the pair such that the cell-cell junction was located between two dam structures, and surrounded the junction region with mineral oil to separate the two compartments. Pulled-glass pipette-tip perfusion and integrated electrodes were used to explore the mechanical and electrical coupling of the cells as well as the transmission of Ca^{+2} waves under a variety of physiological and nonphysiological conditions.

The ability to contain cell populations or specific parts of a cell provides opportunities to focus biochemical influences on specific biological elements and to study the causal relationships among elements. Although a certain level of compartmentalization can be achieved using pipettes, and although pipettes remain a superior method for electrical isolation of different compartments (i.e., patch clamping), microfluidic systems have greatly increased the number of available compartmentalization methods.

4. SYSTEMS FOR CELL-BASED MODELS

4.1. Integrated Analysis

Every cell-based experiment requires a reliable method of extracting information. Thus far, we have considered the ways in which microfluidic systems serve to construct environments that yield improved cellular models. Analytical methods may also be integrated with microfluidic systems to produce information from cell models. Analyses of single mammalian cells in microfluidic systems were recently reviewed by Sims and Allbritton (92), and analyses of cell cultures were described in reviews by Anderson and van den Berg (6) and El-Ali et al. (3).

Many microfluidic cell culture studies have relied on traditional microscopy techniques including phase contrast imaging, histological stains, fluorescent dyes, and immunocytochemistry. Yu et al. employed a plate reader to quantify cell number (93). Thompson et al. studied dynamic gene expression profiles using green fluorescent protein reporters, and King et al. (94) studied the same for multiple genes and stimuli in a multiplexed microfluidic system.

Additionally, several methods rely on PCR. Wilding et al. reported an application of PCR to analyze cells captured in a microfabricated filter (95). Easley et al. presented a microfluidic system integrating all the components necessary to go from a complex biological sample to a final PCR result (96). Marcy et al. demonstrated genomic analysis of complex populations of microbes at the organism level using a microfluidic genome amplification system (97). This integrated microfluidic system contained nine amplification units and allowed selection of individual cells, lysis, sample preparation, and amplification via PCR. The results from this study demonstrate the heterogeneity of biological samples and the future utility of highly parallel microfluidic systems for analysis.

Capillary electrophoresis was one of the first systems to employ microfluidic channels (98), and it has continued to develop high-performance tools for use in various applications, including sequencing (99). Electrophoresis has also been combined with

cell culture systems to identify and quantify the activities of intracellular enzymes (100).

Biosensors have also been employed to study cells in culture. Bratten et al. measured purine release from cardiomyocytes under conditions mimicking cardiac ischemia (101). The authors used a cascade of three enzymes to convert the analyte into hydrogen peroxide that could be detected by the sensor. Chen et al. employed amperometric sensors to detect quantal secretion of catecholamines from individual cells in microfabricated wells (102).

Other demonstrations of integrated analytical tools include cell culture on cantilevers coupled with an atomic force microscopy detection apparatus used to study myotube function (103), application of cylindrical optics to count proteins in single-cell lysates (104), and integrated spectrometry for fluorescence analysis (105).

4.2. Throughput

Microfluidic systems have the potential to increase the biological relevance of cell culture models and to contribute to improved assay content. For large-scale biology research applications (106), as well as for industry applications such as in drug discovery, throughput is an important consideration. Various methods have been used to increase microfluidic system throughput, some of which were discussed in a recent review by Dittrich and Manz (107). The approaches that have been applied to cell culture can be divided into four general categories: (a) microarrays, (b) gradient devices, (c) valved arrays, and (d) individually addressable channel arrays.

Several tools exist for spotting arrays that can be employed to spot different matrices for cell culture or possibly the cells themselves. Microarrays provide a high spatial density of different surface coatings and/or different cell types (78, 108, 109), which represents a great advantage for screening growth surfaces, et cetera. In microarrays, however, all of the cells will be exposed to the same fluid environment. Therefore, they are not suitable for screening soluble factors, including drug compounds.

Gradient devices can provide different concentrations of a single compound for a single cell type. Hung et al. reported the use of a microfluidic gradient generator to address an array of 10×10 cell culture chambers (110). All 10 columns were seeded with the same cell type, and a gradient generator was used to produce a linearly varying concentration across the rows. A similar design was reported by Thompson et al. (111), who used the same inputs for cell seeding and perfusion. A scalable linear gradient generator for cell assays was presented by Walker et al. (112).

Valved arrays enable simultaneous testing of multiple cell types and multiple compounds. With the use of valves, Wang et al. demonstrated multiplexed cytotoxicity testing in a high-density microfluidic array with six distinct cell inputs and 12 distinct compound inputs (113). After 24 h in culture, three different cell lines were exposed to a panel of five toxins. Cell morphology and viability were comparable to those of 96-well control cultures. Using a similar approach for selecting between seeding and perfusion mode, King et al. studied dynamic responses to soluble factor stimuli in an 8×8 array (94).

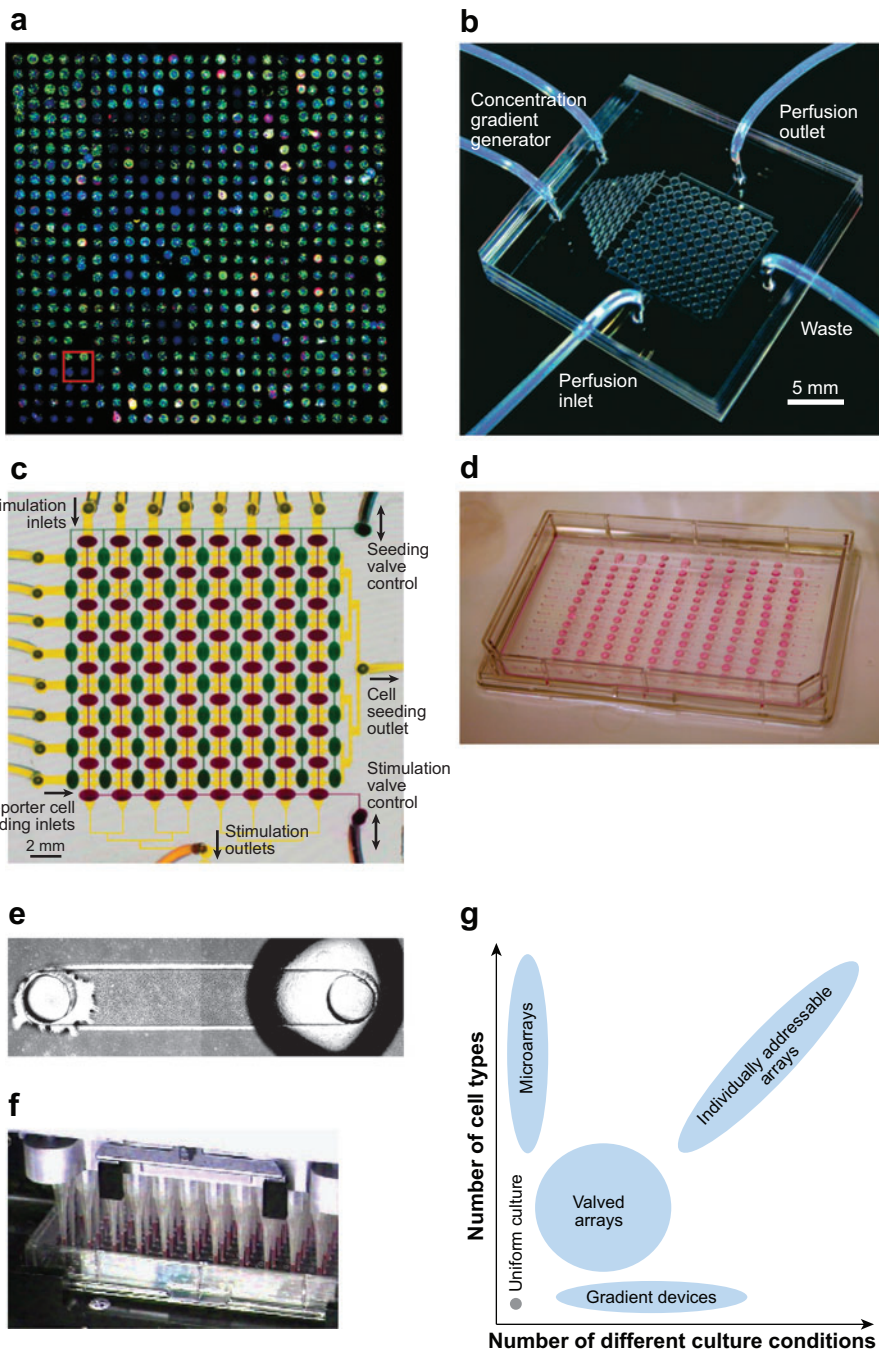
Meyvantsson and Beebe demonstrated cell culture in arrays of 192 individually addressable microchannels (114, 115). Droplet-based passive pumping (116) was employed to seed cells, change media, and treat and stain cells. This approach requires no physical connections between external instruments and the microfluidic device, and is compatible with hand pipettes as well as generic liquid-handling robotics. Warrick et al. analyzed the fluid replacement in these arrays (117) and Berthier et al. presented an analytical model (118) that indicates that perfusion culture will be feasible using passive pumping.

A comparison of different approaches to high-throughput microfluidic cell assays is presented in **Figure 3**. A wide range of flow velocities can be applied in perfusion culture using microarrays, gradient devices, and valved arrays. In channel arrays operated by passive pumping, however, the range is smaller. As mentioned above, the natural perfusion velocity for most cells is very slow and falls within the range available via passive pumping. Whereas microarray cell culture allows screening of a large number of surface bound elements, soluble factors, if any, are uniform across the entire array. The opposite is true for gradient devices, where several different concentrations are possible but where all the cells and other surface-bound elements are identical. Valved arrays represent a compromise between these two methods, and allow several cell types and several culture environments to be assayed simultaneously. However, the constraints imposed by the physical connections required for valved arrays severely limit throughput. In contrast, individually addressable channels can have a large number of access ports (384 ports are shown in **Figure 3d**, and we have demonstrated up to 1536 access ports). When using passive pumping, the density of assay chambers is limited only by the precision of the liquid-handling equipment in its ability to control evaporation in small volumes. Thus, a number of different approaches are being pursued, each of which has inherent advantages and disadvantages that must be taken into account with regard to the intended application.

5. FABRICATION OF MICROFLUIDIC CELL CULTURE DEVICES

Currently, there are several fabrication challenges facing microfluidics researchers. A wide variety of macro-to-micro interfaces exist (119) that need to be standardized (120). Two important aspects of microfluidic interfacing are automation (3) and ease of handling (107), both of which must be considered when designing microfluidic systems.

Also related to interfacing is choice of materials. Many research devices have been made from PDMS (121), and some interfacing strategies have relied on its elastic properties. However, the material properties of PDMS may be problematic for cell culture models (122, 123). Because several molecules involved in cell microenvironments are small and hydrophobic, it is necessary to characterize the effect of PDMS absorption of such molecules upon cell culture in microfluidic devices. Although glass is very common for chemical analysis devices, the cost is too high for disposable



devices. Therefore, thermoplastic materials are the most desirable for microfluidic cell culture devices.

To that end, fabrication of microfluidic structures using thermoplastic materials has been demonstrated (124). However, application in cell culture may impose unique constraints upon materials and fabrication methods. For instance, materials to be used for construction should not leach monomers or additives into the cell culture medium. Similarly, adhesives and solvents should be avoided when choosing a chip-bonding method. In every case, all materials employed in device fabrication must be tested with the cells of intended use.

Other factors to consider when selecting materials include the materials' background fluorescence (125), their ability to produce appropriate surface chemistry for cell attachment (referred to as tissue culture treatment), and their compatibility with standard sterilization methods such as autoclaving, gamma irradiation, ethylene oxide exposure, or low-pressure plasma treatment.

6. SUMMARY AND FUTURE OUTLOOK

Microfluidic cell culture devices enable both basic cell biology research and development of engineered tissues. Careful characterization of cell culture in microfluidic devices is critical in order to understand which macroscale assumptions hold true when culture is scaled down. The number of reports describing microfluidic methods to control cell microenvironments is ever increasing, and these methods are being used to make novel discoveries in cell biology. Despite this trend, we have a long way to go to before we can reproduce *in vivo* microenvironments.

If microfluidic systems can be employed to establish a sufficiently complete microenvironment for human cells in culture, this may lead to *in vitro* models that would outperform both conventional cell culture models and animal models in predicting tissue-specific responses in humans. Such a cell culture platform would greatly enhance basic research in cell biology and would improve our ability to study disease.

An important challenge for the microfluidics community is to find ways of simplifying the use of microfluidic devices. Although the utility of microfluidic devices is becoming increasingly evident, such devices will not be adopted by researchers in

Figure 3

Throughput in microfluidic cell culture. (a) Microarray cell culture. Reprinted with permission from Reference 108. Copyright 2004, Macmillan. (b) Gradient device. Reprinted with permission from Reference 110. Copyright 2005, Wiley-Liss. (c) Valved array. Reprinted with permission from Reference 94. Copyright 2007, Royal Society of Chemistry. (d) Microconduit array using passive pumping to interface an array of individually addressable microchannels. (e) 3T3-L1 cells on day 12 in culture in a microconduit array. (f) A microconduit array interfaced with a generic high-throughput liquid-handling system. (g) A comparison of parallel microfluidic cell culture approaches in terms of number of different cell types and culture conditions achievable.

medicine or the life sciences unless they can be used off the shelf without the aid of engineers.

DISCLOSURE STATEMENT

The authors have an ownership interest in Bellbrook Labs, LLC, which has licensed technology presented in this review.

ACKNOWLEDGMENTS

The authors would like to thank members of the University of Wisconsin, Madison MMB laboratory for their support. **Figure 3a** is reprinted with permission from Macmillan Publishers Ltd. **Figure 3b** is reprinted with permission from Wiley-Liss, Inc., a subsidiary of John Wiley & Sons, Inc. **Figure 3c** is reprinted with permission from the Royal Society of Chemistry.

LITERATURE CITED

1. Harrison R. 1907. Observations on the living developing nerve fiber. *Anat. Rec.* 1:116–28
2. Voldman J, Gray ML, Schmidt MA. 1999. Microfabrication in biology and medicine. *Annu. Rev. Biomed. Eng.* 1:401–25
3. El-Ali J, Sorger PK, Jensen KF. 2006. Cells on chips. *Nature* 442:403–11
4. Folch A, Toner M. 2000. Microengineering of cellular interactions. *Annu. Rev. Biomed. Eng.* 2:227–56
5. Beebe DJ, Mensing GA, Walker GM. 2002. Physics and applications of microfluidics in biology. *Annu. Rev. Biomed. Eng.* 4:261–86
6. Anderson H, van den Berg A. 2003. Microfluidic devices for cellomics: a review. *Sens. Actuators B* 92:315–25
7. Sawano A, Takayama S, Matsuda M, Miyawaki A. 2002. Lateral propagation of EGF signaling after local stimulation is dependent on receptor density. *Dev. Cell* 3:245–57
8. Lucchetta EM, Lee JH, Fu LA, Patel NH, Ismagilov RF. 2005. Dynamics of *Drosophila* embryonic patterning network perturbed in space and time using microfluidics. *Nature* 434:1134–38
9. Thery M, Racine V, Pepin A, Piel M, Chen Y, et al. 2005. The extracellular matrix guides the orientation of the cell division axis. *Nat. Cell Biol.* 7:947–57
10. Chen CS, Mrksich M, Huang S, Whitesides GM, Ingber DE. 1997. Geometric control of cell life and death. *Science* 276:1425–28
11. Whitesides GM, Ostuni E, Takayama S, Jiang X, Ingber DE. 2001. Soft lithography in biology and biochemistry. *Annu. Rev. Biomed. Eng.* 3:335–73
12. Park TH, Shuler ML. 2003. Integration of cell culture and microfabrication technology. *Biotechnol. Prog.* 19:243–53
13. Walker GM, Zeringue HC, Beebe DJ. 2004. Microenvironment design considerations for cellular scale studies. *Lab Chip* 4:91–97

14. Atencia J, Beebe DJ. 2005. Controlled microfluidic interfaces. *Nature* 437:648–55
15. Kim L, Toh Y, Voldman J, Yu H. 2007. A practical guide to microfluidic perfusion culture of adherent mammalian cells. *Lab Chip* 7:681–94
16. Tilles AW, Baskaran H, Roy P, Yarmush ML, Toner M. 2001. Effects of oxygenation and flow on the viability and function of rat hepatocytes cocultured in a microchannel flat-plate bioreactor. *Biotechnol. Bioeng.* 73:379–89
17. Leclerc E, Sakai Y, Fujii T. 2003. Cell culture in a three-dimensional network of PDMS (polydimethylsiloxane) microchannels. *Biomed. Microdev.* 5:109–14
18. Yu H, Meyvantsson I, Shkel IA, Beebe DJ. 2005. Diffusion dependent cell behavior in microenvironments. *Lab Chip* 5:1089–95
19. Yu H, Alexander CM, Beebe DJ. 2007. Understanding microchannel culture: parameters involved in soluble factor signaling. *Lab Chip* 7:726–30
20. Raty S, Walters EM, Davis J, Zeringue H, Beebe DJ, et al. 2004. Embryonic development in the mouse is enhanced via microchannel culture. *Lab Chip* 4:186–90
21. Dolberg DS, Bissell MJ. 1984. Inability of Rous sarcoma virus to cause sarcomas in the avian embryo. *Nature* 309:552–56
22. Abbott A. 2003. Cell culture: biology's new dimension. *Nature* 424:870–72
23. Toh YC, Zhang C, Zhang J, Khong YM, Chang S, et al. 2007. A novel 3D mammalian cell perfusion–culture system in microfluidic channels. *Lab Chip* 7:302–9
24. Kim MS, Yeon JH, Park JK. 2007. A microfluidic platform for 3-dimensional cell culture and cell-based assays. *Biomed. Microdev.* 9:25–34
25. Paguirigan A, Beebe DJ. 2006. Gelatin-based microfluidic devices for cell culture. *Lab Chip* 6:407–13
26. Udy GB, Towers RP, Snell RG, Wilkins RJ, Park SH, et al. 1997. Requirement of STAT5b for sexual dimorphism of body growth rates and liver gene expression. *Proc. Natl. Acad. Sci. USA* 94:7239–44
27. Yan Y, Yang D, Zarnowska ED, Du Z, Werbel B, et al. 2005. Directed differentiation of dopaminergic neuronal subtypes from human embryonic stem cells. *Stem Cells* 23:781–90
28. Davenport RJ. 2005. What controls organ regeneration? *Science* 309:84
29. Blaxter M. 1998. *Caenorhabditis elegans* is a nematode. *Science* 282:2041–46
30. Wu D. 2005. Signaling mechanisms for regulation of chemotaxis. *Cell Res.* 15:52–56
31. Martin P. 1997. Wound healing: aiming for perfect skin regeneration. *Science* 276:75–81
32. Mackenzie IC, Fusenig NE. 1983. Regeneration of organized epithelial structure. *J. Invest. Dermatol.* 81:189–94
33. Jungermann K, Kietzmann T. 2000. Oxygen: modulator of metabolic zonation and disease of the liver. *Hepatology* 31:255–60
34. Montesano R, Pepper MS, Orci L. 1993. Paracrine induction of angiogenesis in vitro by Swiss 3T3 fibroblasts. *J. Cell Sci.* 105:1013–24
35. Tabata T, Takei Y. 2004. Morphogens, their identification and regulation. *Development* 131:703–12

36. Blake AJ, Pearce TM, Rao NS, Johnson SM, Williams JC. 2007. Multilayer PDMS microfluidic chamber for controlling brain slice microenvironment. *Lab Chip* 7:842–49
37. Jeon NL, Baskaran H, Dertinger SKW, Whitesides GM, Van de Water L, Toner M. 2002. Neutrophil chemotaxis in linear and complex gradients of interleukin-8 formed in a microfabricated device. *Nat. Biotechnol.* 20:826–30
38. Wang S-J, Saadi W, Lin F, Nguyen CM-C, Jeon NL. 2004. Differential effect of EGF gradient profiles on breast cancer cell chemotaxis. *Exp. Cell Res.* 300:180–89
39. Irimia D, Liu SY, Tharp WG, Samadani A, Toner M, Poznansky MC. 2006. Microfluidic system for measuring neutrophil migratory responses to fast switches of chemical gradients. *Lab Chip* 6:191–98
40. Mao H, Cremer PS, Manson MD. 2003. A sensitive, versatile microfluidic assay for bacterial chemotaxis. *Proc. Natl. Acad. Sci. USA* 100:5449–54
41. Chung BG, Flanagan LA, Rhee SW, Schwartz PH, Lee AP, et al. 2005. Human neural stem cell growth and differentiation in a gradient-generating microfluidic device. *Lab Chip* 5:401–6
42. Walker GM, Sai J, Richmond A, Stremmer M, Chung CY, Wikswo JP. 2005. Effects of flow and diffusion on chemotaxis studies in a microfabricated gradient generator. *Lab Chip* 5:611–18
43. Berthier ES, Warrick JW, Yu H, Beebe DJ. 2008. Managing evaporation for more robust microscale assays. Part 2: Characterization of convection and diffusion for cell biology. *Lab Chip*. doi: 10.1039/b717423c
44. Abhyankar VV, Lokuta MA, Huttenlocher A, Beebe DJ. 2006. Characterization of a membrane-based gradient generator for use in cell-signaling studies. *Lab Chip* 6:389–93
45. Wu HK, Huang B, Zare RN. 2006. Generation of complex, static solution gradients in microfluidic channels. *J. Am. Chem. Soc.* 128:4194–95
46. Rosoff WJ, McAllister R, Esrick MA, Goodhill GJ, Urbach JS. 2005. Generating controlled molecular gradients in 3D gels. *Biotechnol. Bioeng.* 91:754–59
47. Cheng S, Heilman S, Wasserman M, Archer S, Shuler ML, Wu M. 2007. A hydrogel-based microfluidic device for the studies of directed cell migration. *Lab Chip* 7:763–69
48. Griffith LG, Swartz MA. 2006. Capturing complex 3D tissue physiology in vitro. *Nat. Rev. Mol. Cell Biol.* 7:211–24
49. Lutolf MP, Hubbell JA. 2005. Synthetic biomaterials as instructive extracellular microenvironments for morphogenesis in tissue engineering. *Nat. Biotechnol.* 23:47–55
50. Dertinger SKW, Jiang X, Li Z, Murthy VN, Whitesides GM. 2002. Gradients of substrate-bound laminin orient axonal specification of neurons. *Proc. Natl. Acad. Sci. USA* 99:12542–47
51. Tan W, Desai TA. 2004. Microscale multilayer cocultures for biomimetic blood vessels. *J. Biomed. Mater. Res.* 72A:146–60
52. Frisk T, Rydholm S, Andersson H, Stemme G, Brismar H. 2005. A concept for miniaturized 3-D cell culture using an extracellular matrix gel. *Electrophoresis* 26:4751–58

53. Evans AR, Euteneuer S, Chavez E, Mullen LM, Hui EE, et al. 2007. Laminin and fibronectin modulate inner ear spiral ganglion neurite outgrowth in an in vitro alternate choice assay. *Dev. Neurobiol.* 67:1721–30
54. Tsang VL, Chen AA, Cho LM, Jadin KD, Sah RL, et al. 2007. Fabrication of 3D hepatic tissues by additive photopatterning of cellular hydrogels. *FASEB J.* 21:790–801
55. Underhill GH, Chen AA, Albrecht DR, Bhatia SN. 2007. Assessment of hepatocellular function within PEG hydrogels. *Biomaterials* 28:256–70
56. Sud D, Mehta G, Mehta K, Linderman J, Takayama S, Mycek MA. 2006. Optical imaging in microfluidic bioreactors enables oxygen monitoring for continuous cell culture. *J. Biomed. Opt.* 11:050504
57. Allen JW, Bhatia SN. 2003. Formation of steady-state oxygen gradients in vitro: application to liver zonation. *Biotechnol. Bioeng.* 82:253–62
58. Allen JW, Khetani SR, Bhatia SN. 2005. In vitro zonation and toxicity in a hepatocyte bioreactor. *Toxicol. Sci.* 84:110–19
59. Allen JW, Khetani SR, Johnson RS, Bhatia SN. 2006. In vitro liver tissue model established from transgenic mice: role of HIF-1 α on hypoxic gene expression. *Tissue Eng.* 12:3135–47
60. Park J, Bansal T, Pinelis M, Maharbiz MM. 2006. A microsystem for sensing and patterning oxidative microgradients during cell culture. *Lab Chip* 6:611–22
61. Pugh CW, Radcliffe PJ. 2003. Regulation of angiogenesis by hypoxia: role of the HIF system. *Nat. Med.* 9:677–84
62. Li YS, Haga JH, Chien S. 2005. Molecular basis of the effects of shear stress on vascular endothelial cells. *J. Biomech.* 38:1949–71
63. Davis MJ, Hill MA. 1999. Signaling mechanisms underlying the vascular myogenic response. *Physiol. Rev.* 79:387–423
64. Widmaier EP, Raff H, Strang KT. 2004. *Human Physiology*, ed. SI Fox, pp. 375–466. New York: McGraw-Hill. 9th ed.
65. Leclerc E, David B, Griscom L, Lepioufle B, Fujii T, et al. 2006. Study of osteoblastic cells in a microfluidic environment. *Biomaterials* 27:586–95
66. Figallo E, Cannizzaro C, Gerecht S, Burdick JA, Langer R, et al. 2007. Microbioreactor array for controlling cellular microenvironments. *Lab Chip* 7:710–19
67. Schaff UY, Xing MM, Lin KK, Pan N, Jeon NL, Simon SI. 2007. Vascular mimetics based on microfluidics for imaging the leukocyte–endothelial inflammatory response. *Lab Chip* 7:448–56
68. Discher DE, Janmey P, Wang YL. 2005. Tissue cells feel and respond to the stiffness of their substrate. *Science* 310:1139–43
69. Li S, Guan JL, Chien S. 2005. Biochemistry and biomechanics of cell motility. *Annu. Rev. Biomed. Eng.* 7:105–50
70. Ingber DE. 2003. Tensegrity I. Cell structure and hierarchical systems biology. *J. Cell Sci.* 116:1157–73
71. Ingber DE. 2003. Tensegrity II. How structural networks influence cellular information processing networks. *J. Cell Sci.* 116:1397–408
72. Wozniak MA, Desai R, Solski PA, Der CJ, Keely PJ. 2003. ROCK-generated contractility regulates breast epithelial cell differentiation in response to the

- physical properties of a three-dimensional collagen matrix. *J. Cell Biol.* 163:583–95
73. McBeath R, Pirone DM, Nelson CM, Bhadriraju K, Chen CS. 2004. Cell shape, cytoskeletal tension, and RhoA regulate stem cell lineage commitment. *Dev. Cell* 6:483–95
74. Balaban NQ, Schwarz US, Riveline D, Goichberg P, Tzur G, et al. 2001. Force and focal adhesion assembly: a close relationship studied using elastic micropatterned substrates. *Nat. Cell Biol.* 3:466–72
75. Tan JL, Tien J, Pirone DM, Gray DS, Bhadriraju K, Chen CS. 2003. Cells lying on a bed of microneedles: an approach to isolate mechanical force. *Proc. Natl. Acad. Sci. USA* 100:1484–89
76. Nelson CM, Van Duijn MM, Inman JL, Fletcher DA, Bissell MJ. 2006. Tissue geometry determines sites of mammary branching morphogenesis in organotypic cultures. *Science* 314:298–300
77. Torisawa Y, Chueh BH, Huh D, Ramamurthy P, Roth TM, et al. 2007. Efficient formation of uniform-sized embryoid bodies using a compartmentalized microchannel device. *Lab Chip* 7:770–76
78. Karp JM, Yeh J, Eng G, Fukuda J, Blumling J, et al. 2007. Controlling size, shape and homogeneity of embryoid bodies using poly(ethylene glycol) microwells. *Lab Chip* 7:786–94
79. Powers MJ, Domansky K, Kaazempur-Mofrad MR, Kalezi A, Capitano A, et al. 2002. A microfabricated array bioreactor for perfused 3D liver culture. *Biotechnol. Bioeng.* 78:257–69
80. Gottwald E, Giselbrecht S, Augspurger C, Lahni B, Dambrowsky N, et al. 2007. A chip-based platform for the in vitro generation of tissues in three-dimensional organization. *Lab Chip* 7:777–85
81. Kastrup CJ, Shen F, Runyon MK, Ismagilov RF. 2007. Characterization of the threshold response of initiation of blood clotting to stimulus patch size. *Biophys. J.* 93:2969–77
82. Hui EE, Bhatia SN. 2007. Micromechanical control of cell-cell interactions. *Proc. Natl. Acad. Sci. USA* 104:5722–26
83. Tao S, Young C, Redenti S, Zhang Y, Klassen H, et al. 2007. Survival, migration and differentiation of retinal progenitor cells transplanted on micro-machined poly(methyl methacrylate) scaffolds to the subretinal space. *Lab Chip* 7:695–701
84. Sonna LA, Fujita J, Gaffin SL, Lilly CM. 2002. Invited review: Effects of heat and cold stress on mammalian gene expression. *J. Appl. Physiol.* 92:1725–42
85. Pearce TM, Wilson JA, Oakes SG, Chiu SY, Williams JC. 2005. Integrated microelectrode array and microfluidics for temperature clamp of sensory neurons in culture. *Lab Chip* 5:97–101
86. Thorsen T, Maerkl SJ, Quake SR. 2002. Microfluidic large-scale integration. *Science* 298:580–84
87. Groisman A, Lobo C, Cho H, Campbell JK, Dufour YS, et al. 2005. A microfluidic chemostat for experiments with bacterial and yeast cells. *Nat. Methods* 2:685–49

88. Balagaddé FK, You L, Hansen CL, Arnold FH, Quake SR. 2005. Long-term monitoring of bacteria undergoing programmed population control in a microchemostat. *Science* 309:137–40
89. Taylor AM, Blurton-Jones M, Rhee SW, Cribbs DH, Cotman CW, Jeon NL. 2005. A microfluidic culture platform for CNS axonal injury, regeneration and transport. *Nat. Methods* 2:599–605
90. Ravula SK, Wang MS, McClain MA, Asress SA, Frazier B, Glass JD. 2007. Spatiotemporal localization of injury potentials in DRG neurons during vincristine-induced axonal degeneration. *Neurosci. Lett.* 415:34–39
91. Klauke N, Smith G, Cooper JM. 2007. Microfluidic systems to examine intercellular coupling of pairs of cardiac myocytes. *Lab Chip* 7:731–39
92. Sims CE, Allbritton NL. 2007. Analysis of single mammalian cells on-chip. *Lab Chip* 7:423–40
93. Yu H, Alexander CM, Beebe DJ. 2007. A platereader-compatible microchannel array for cell biology assays. *Lab Chip* 7:388–91
94. King KR, Wang S, Irimia D, Jayaraman A, Toner M, Yarmush ML. 2007. A high-throughput microfluidic real-time gene expression living cell array. *Lab Chip* 7:77–85
95. Wilding P, Kricka LJ, Cheng J, Hvichia G, Shoffner MA, Fortina P. 1998. Integrated cell isolation and polymerase chain reaction analysis using silicon microfilter chambers. *Anal. Biochem.* 257:95–100
96. Easley CJ, Karlinsey JM, Bienvenue JM, Legendre LA, Roper MG, et al. 2006. A fully integrated microfluidic genetic analysis system with sample-in-answer-out capability. *Proc. Natl. Acad. Sci. USA* 103:19272–77
97. Marcy Y, Ouverney C, Bik EM, Lösekann T, Ivanova N, et al. 2007. Dissecting biological “dark matter” with single-cell genetic analysis of rare and uncultivated TM7 microbes from the human mouth. *Proc. Natl. Acad. Sci. USA* 104:11889–94
98. Harrison DJ, Fluri K, Seiler K, Fan Z, Effenhauser CS, Manz A. 1993. Micromachining a miniaturized capillary electrophoresis-based chemical analysis system on a chip. *Science* 261:895–97
99. Aborn JH, El-Difrawy SA, Novotny M, Gismondi EA, Lam R, et al. 2005. A 768-lane microfabricated system for high-throughput DNA sequencing. *Lab Chip* 5:669–74
100. Meredith GD, Sims CE, Soughayer JS, Allbritton NL. 2000. Measurement of kinase activation in single mammalian cells. *Nat. Biotechnol.* 18:309–12
101. Bratten CDT, Cobbold PH, Cooper JM. 1998. Single-cell measurements of purine release using a micromachined electroanalytical sensor. *Anal. Chem.* 70:1164–70
102. Chen P, Xu B, Tokranova N, Feng X, Castracane J, Gillis KD. 2003. Amperometric detection of quantal catecholamine secretion from individual cells on micromachined silicon chips. *Anal. Chem.* 75:518–24
103. Wilson K, Molnar P, Hickman J. 2007. Integration of functional myotubes with a Bio-MEMS device for non-invasive interrogation. Integration of functional myotubes with a Bio-MEMS device for non-invasive interrogation. *Lab Chip* 7:920–22

104. Huang B, Wu H, Bhaya D, Grossman A, Granier S, et al. 2007. Counting low-copy number proteins in a single cell. *Science* 315:81–84
105. Schmidt O, Bassler M, Kiesel P, Knollenberg C, Johnson N. 2007. Fluorescence spectrometer-on-a-fluidic-chip. *Lab Chip* 7:626–29
106. Abraham VC, Taylor DL, Haskins JR. 2004. High content screening applied to large-scale cell biology. *Trends Biotechnol.* 22:15–22
107. Dittrich PS, Manz A. 2006. Lab-on-a-chip: microfluidics in drug discovery. *Nat. Rev. Drug Discov.* 5:210–18
108. Anderson DG, Levenberg S, Langer R. 2004. Nanoliter-scale synthesis of arrayed biomaterials and application to human embryonic stem cells. *Nat. Biotechnol.* 22:863–66
109. Flaim CJ, Chien S, Bhatia SN. 2005. An extracellular matrix microarray for probing cellular differentiation. *Nat. Methods* 2:119–25
110. Hung PJ, Lee PJ, Sabounchi P, Lin R, Lee LP. 2005. Continuous perfusion microfluidic cell culture array for high-throughput cell-based assays. *Biotechnol. Bioeng.* 89:1–8
111. Thompson DM, King KR, Wieder KJ, Toner M, Yarmush ML, Jayaraman A. 2004. Dynamic gene expression profiling using a microfabricated living cell array. *Anal. Chem.* 76:4098–103
112. Walker GM, Monteiro-Riviere N, Rouse J, O'Neill AT. 2007. A linear dilution microfluidic device for cytotoxicity assays. *Lab Chip* 7:226–32
113. Wang Z, Kin M, Marquez M, Thorsen T. 2007. High-density microfluidic arrays for cell cytotoxicity analysis. *Lab Chip* 7:740–45
114. Meyvantsson I, Beebe DJ. 2005. High throughput microfluidic system for heterogeneous assays. *Proc. Annu. Int. IEEE/EMBS Conf. Microtech. Med. Biol., 3rd, Oahu*, pp. 42–44. Piscataway: IEEE
115. Meyvantsson I, Warrick JW, Hayes S, Skoien A, Beebe DJ. 2008. Automated cell culture in high density tubeless microfluidic device arrays. *Lab Chip*. doi: 10.1039/b71537a
116. Walker GM, Beebe DJ. 2002. A passive pumping method for microfluidic devices. *Lab Chip* 2:131–34
117. Warrick JW, Meyvantsson I, Ju J, Beebe DJ. 2007. High-throughput microfluidics: improved sample treatment and washing over standard wells. *Lab Chip* 7:316–21
118. Berthier ES, Berthier JC, Beebe DJ. 2007. Flow rate analysis of a surface tension driven micropump. *Lab Chip* 7:1475–78
119. Fredrickson CK, Fan ZH. 2004. Macro-to-micro interfaces for microfluidic devices. *Lab Chip* 4:526–33
120. Whitesides GM. 2006. The origins and the future of microfluidics. *Nature* 442:368–73
121. Duffy D, McDonald J, Schueller O, Whitesides GM. 1998. Rapid prototyping of microfluidic systems in poly(dimethylsiloxane). *Anal. Chem.* 70:4974–84
122. Toepke MW, Beebe DJ. 2006. PDMS absorption of small molecules and consequences in microfluidic applications. *Lab Chip* 6:1484–86

123. Mukhopadhyay R. 2007. When PDMS isn't the best: What are its weaknesses, and which other polymers can researchers add to their toolboxes? *Anal. Chem.* 79:3248–53
124. Becker H, Gärtner C. 2000. Polymer microfabrication methods for microfluidic analytical applications. *Electrophoresis* 21:12–26
125. Hawkins KR, Yager P. 2003. Nonlinear decrease of background fluorescence in polymer thin-films: a survey of materials and how they can complicate fluorescence detection in microTAS. *Lab Chip* 3:248–52