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Microfluidic cell culture

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Microfluidic techniques allow precise control of fluids and particles at the nanoliter scale and facilitate simultaneous manipulation and analysis of cultured cells, starting from a single cell to larger populations and to intact tissues. The use of integrated microfluidic devices has considerably advanced the fields of quantitative and systems biology. In this review, we survey the recent developments in microfluidic cell culture, and discuss not only the advantages but also limitations of using such systems, and give an outlook on potential future developments.

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Introduction

The establishment of techniques to maintain and grow cells *in vitro* is a major milestone in biological sciences. Since its introduction in 1912 [1], methods have been developed for culturing, expanding, differentiating and de-differentiating cells [2]. The basic tools and requirements for cell culture (Table 1) have not changed significantly since then. Besides substantial advances in controlling for contamination with bacteria, mold and yeast, most improvements in cell-culture have been made to the culture media and the materials used for production of the cell culture dish. Interestingly, even the shape of the cell culture dish remained mostly unchanged for nearly a century. The recent use of robotics to eliminate time-consuming manual pipetting steps resulted in increased throughput and accuracy, and constitutes a rare yet exceptional improvement of the technique. Despite their bulky and complicated nature and the substantial costs associated with their use, robotic systems have now become a cornerstone of large-scale industrial applications of cell culture. Nevertheless, the principles of traditional cell culture techniques remain mostly unchanged by these modifications.

Recent insight from the emerging fields of quantitative and systems biology highlighted the importance of analyzing individual cells instead of just bulk cultures. Because of natural cell-to-cell variability in biochemical parameters such as mRNA and protein expression levels, and the inevitable stochastic molecular noise, population-averaged bulk assays are often inaccurate or misleading. Further, cellular biochemical parameters and signaling states constantly change, making dynamical analysis of cells crucial in understanding how biological systems operate. Basic methods in single-cell and dynamical analysis have mostly been limited to conventional cell culture techniques using the traditional dish and the hand-held pipette. Recent introduction of microfluidics to biological sciences allowed addressing these fundamental limitations (Table 1). Microfluidic cell culture allows controlling fluid flow in the micrometer and nanoliter scale in precisely defined geometries and facilitates simultaneous manipulation and analysis starting from a single cell level, to larger cell populations and up to tissues cultured on fully integrated and automated chips.

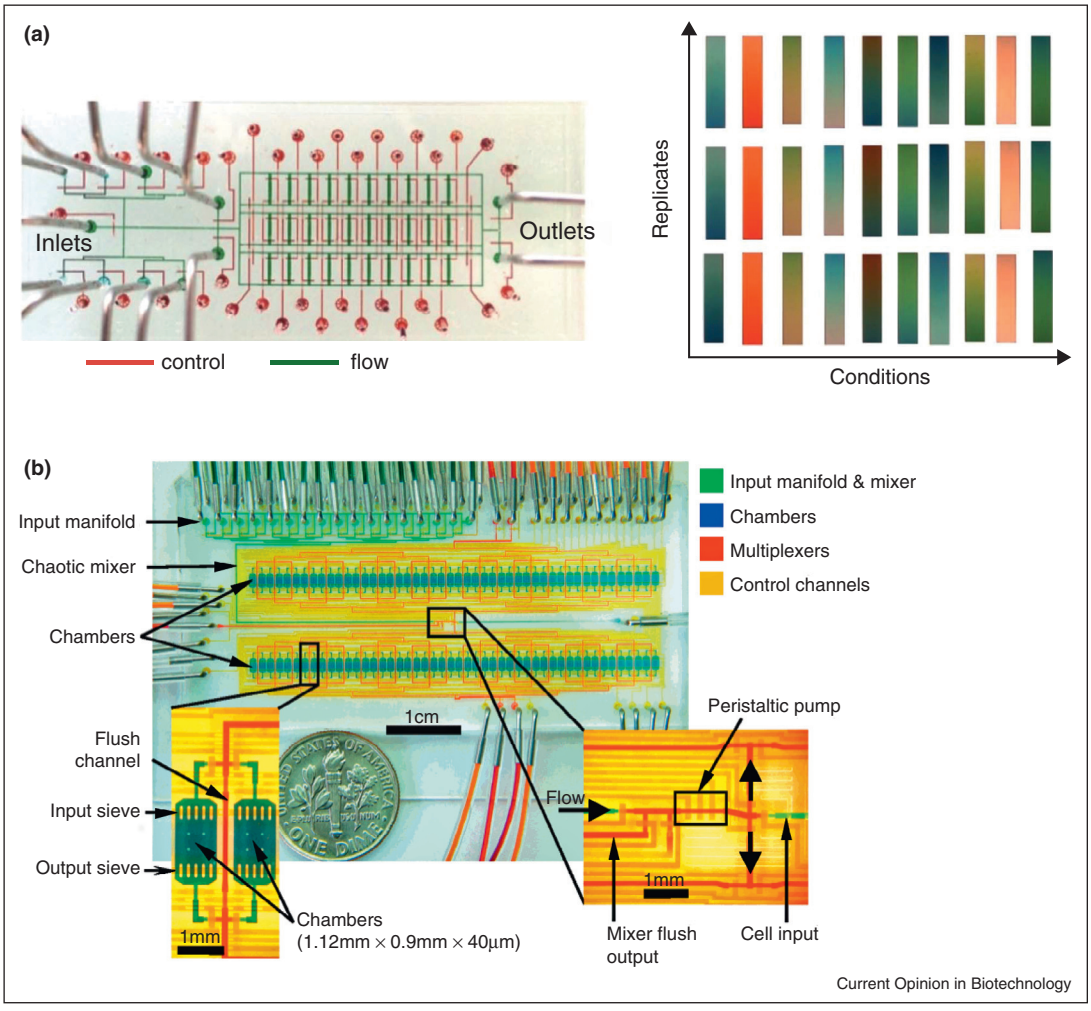
Among the multitude of approaches for manufacturing microfluidic devices, soft lithography of poly-dimethylsiloxane (PDMS) has become standard for cell culture applications. With this technique, structures of micrometer resolution are molded from a hard master into PDMS. Many advanced microfluidic chips use miniaturized micromechanical membrane valves made from PDMS to efficiently manipulate fluids at the microliter scale. Comparable to transistors in electronic devices, these valves allow exact spatial and temporal control of fluid flow and delivery of media, drugs and signaling factors to live cells. Parallelized fabrication using optical lithography and careful alignment of flow and control layers allow rapid construction of various types of channels, chambers and valves in fully integrated, compact devices (Figure 1) [3]. The advent of PDMS micromechanical valves along with multiplexing methods allowed the development of truly 'lab-on-a-chip devices' that improve the accuracy and throughput of biological assays by orders of magnitude. The time from computerized chip design to actual laboratory use of PDMS chips is typically a few weeks. Integration of thousands of micromechanical valves in compact platforms and their automation allows performing tedious, manual-labor intensive tasks efficiently, rapidly and with minimal intervention [4]. The use of nanoliter-sized assay chambers results in increased precision, and reduced consumption of costly reagents. Microfluidics offers the possibility to deliver not only chemical but also mechanical signals, providing an extra degree of control over cultured cells. Examples for

Table 1

Basic requirements for cell culture, and improvements when microfluidic methods are used

Requirements	Conventional cell culture	Microfluidic cell culture
Control of temperature and gasses	Large fluid volumes prevent fast changes	Small volumes allow dynamic control
Addition of nutrients and removal of metabolites	Infrequent, manual exchange of large volumes	Precisely measured, continuous or transient exchange of media
Stimulation with drugs/proteins and simultaneous imaging	Mostly not feasible	Feasible
Parallelization of cellular assays	Not feasible	High capability for parallelization
Automation of cell culture tasks	Bulky, expensive fluid-handling robots must be used	High capability for automation in compact, inexpensive format
Single-cell manipulation and analysis	Manually involved, inaccurate, low throughput	Accurate and high-throughput

Figure 1



Examples of integrated microfluidic cell culture devices fabricated by soft lithography of poly-dimethylsiloxane (PDMS). **(a)** Device for the generation of diffusion-based chemical gradients containing 30 parallel cell-culture chambers (rectangular green structures), and examples of simultaneously generated dye gradients in these chambers (right-sided diagram); adapted from [18•] and reproduced by permission of The Royal Society of Chemistry. **(b)** A high-throughput automated cell culture system with integrated multiplexer, peristaltic pump, cell inlet and waste output. Adapted with permission from [16]. Copyright (2007) American Chemical Society.

mechanical signals are gradients of the surface-stiffness [5] of cell culture devices or exact regulation of shear stress forces [6].

In this review we discuss firstly the advantages, secondly the issues and troubleshooting of the use of microfluidics in cell culture, thirdly the recent developments of microfluidic cell culture techniques, and finally an outlook on open questions and potential developments in the field.

Advantages of microfluidic cell culture

Microfluidics provide high degree of control over cell culture conditions in various aspects (Table 1). Given the small geometrical dimensions in the microscale and nanoscale, the movement of fluids is laminar, and placement of fluid volumes in the nL-range, pL-range and even fL-range is possible [7]. The ability of exactly timing fluid flow using in-chip membrane valves allows precise chemical and physical control of the microenvironment (the response time of valves can be as short as 1 ms). The doses delivered to cells can be measured in nanoliters to femtoliters, representing a significant improvement in precision compared to the traditional pipette that can measure microliters at best. Examples are controlling of glucose [8] and oxygen concentrations [9] with the potential to characterize cellular responses of single cells to these changes. Microfluidic cell culture devices also allow precise control of cell numbers and density in a given area or volume, and can provide placement of cells in complex geometries [10], their monitoring with high spatial and temporal resolution and their individual retrieval during or following experiments. Further, cells can be organized into three-dimensional geometries in matrices such as hydrogels, allowing culture of cells in structures resembling those in tissues [11].

The small dimensions of spatially separated microfluidic compartments allow assembly of a multitude of individually controllable cell culture chambers on a single device. This facilitates high parallelization of experiments, high throughput of samples and reactions and thus improvement of reproducibility, as well as a reduction in reagent costs [12,13].

Stem cells that are difficult to culture with conventional techniques can be expanded relatively rapidly in microfluidic culture [14]. Parallelization of experimental conditions allows for enhanced cell-based screening assays, such as immunophenotyping assays monitoring single cell cytokine production in response to external stimuli [15].

Microfluidic systems can be automated to an immense extent. Automation of microfluidic cell culture systems allows culturing cells for several weeks under precisely defined conditions without manual intervention [7,16]. Non-dividing or slowly dividing cells can be cultured continuously in the same cell culture chamber by regular or constant replacement of media. Automation of cell culture systems leads to standardized manipulation, monitoring and sampling of cultured cells. This allows strict adherence to the timing of protocols, which is of particular value when dynamic processes in smaller time intervals, for example, seconds or minutes, are characterized.

PDMS based microfluidics provide excellent live cell imaging conditions as PDMS offers transparency and stable optical features, and the optical aberrations and auto-fluorescence induced by small volumes of cell culture medium in such devices are generally negligible. Efficient tracking of migrating cells [17••] and phenotyping of resting cells in response to predefined stimuli have been demonstrated. In combination with fluorescent live cell imaging, microfluidic cell culture devices therefore allow powerful characterization of a multitude of cellular responses on a single cell as well as population level.

Resulting from the above-mentioned advantages (controllability, parallelization, automation, excellent imaging properties), microfluidics has become particularly valuable for analysis of single cell dynamics. With the help of microfluidic devices cell growth and regulation of cell size can be directly observed [8,18•,19,20•] and lineages of single cells can be tracked for several generations [21–23]. On a molecular level microfluidics allow the characterization of transcription factor and gene expression dynamics in single-cells thereby adding substantially to our understanding of the function of biological systems [24–26]. Further, the dynamics of protein secretion [27••,28] and the dynamic analysis of signaling pathways have been addressed with the help of microfluidic cell culture devices [24].

Issues and troubleshooting with PDMS based microfluidic cell culture systems

PDMS offers many advantages for manufacturing microfluidic cell culture systems. It allows implementation of robust geometric structures and pneumatic membrane valves and has a low level of auto-fluorescence and is transparent. However, PDMS is a hydrophobic and porous material, which results in the absorption of hydrophobic molecules such as lipids or small molecules [29] from culture media into PDMS (Table 2). To maintain stable cell culture conditions and to reduce the effects of absorption into PDMS, regular replacement of culture media is necessary. Pretreatment of PDMS devices with sol–gel chemistry can reduce small-molecule absorption [30]. Also, small volumes of media in microfluidic cell culture devices can result in faster consumption of nutrients and in an increase of the concentration of metabolites or secreted molecules, especially compared to

Table 2

Major issues with PDMS microfluidic cell culture devices and troubleshooting

Issues	Potential solutions
Absorption of hydrophobic molecules into PDMS	Sol-gel methods to block PDMS pores
PDMS water permeability and evaporation lead to reduction of culture medium	Use of humidifiers or integrated media baths
PDMS toxicity	Extended postproduction baking, autoclaving of devices, or chemical extraction of uncrosslinked species
Insufficient cell adhesion	Pretreatment with adhesion molecules like fibronectin

conventional cell culture techniques. Although these small volume effects need to be carefully considered when designing experimental setups, they arguably reflect physiological conditions of cells or cell-populations in tissues more appropriately than cells that are cultured in larger volumes (Figure 2). However, the small volumes might necessitate more frequent replacement of media or addition of nutrients. Despite the hydrophobic nature of PDMS, its porosity and permeability to gasses and fluids can result in rapid evaporation. This effect can be limited by placing the device in an environment with high humidity, for example, by using incubators connected to a humidifier, or by building on-chip media reservoirs to eliminate evaporation effects [20•]. Integrated incubation systems also allow controlling temperature and the concentrations of oxygen and carbon dioxide. PDMS that is not fully cured can be toxic to cells, and this toxicity is even more pronounced for 'off-ratio' mixtures used when fabricating multi-layer devices [3]. To avoid toxicity, chips should be baked at curing temperatures for extended durations to fully crosslink PDMS [3]. They can also be autoclaved to further improve biological compatibility. Deformation of PDMS due to baking at high temperatures or autoclaving can destroy delicate structures within microfluidic devices, but this can be prevented by careful titration and standardization of the postproduction baking times. Other methods to make PDMS devices more inert and cell-friendly include various chemical extraction methods that significantly improved the culture of primary neurons on PDMS [31]. Cells do not typically attach to native PDMS, and the adhesion of cells to PDMS can be accomplished by coating it with proteins such as fibronectin, or various mixtures such as laminin or matrigel. Cell attachment can induce issues such as congestion of channels and malfunctioning of valves. Pretreatment with protective compounds such as non-ionic triblock copolymers reduces the adhesion of cells at undesired locations. Attention should also be paid to potential flow shearing of cells in confined channels in microfluidic chips. This effect can be limited by reducing flow velocities. Cells can take up PDMS when cultured for longer periods in PDMS-containing microfluidic devices [32] and altered growth characteristics of cells have been observed [33]. These issues warrant consideration when conceptualizing cell culture experiments in microfluidic devices.

Figure 2

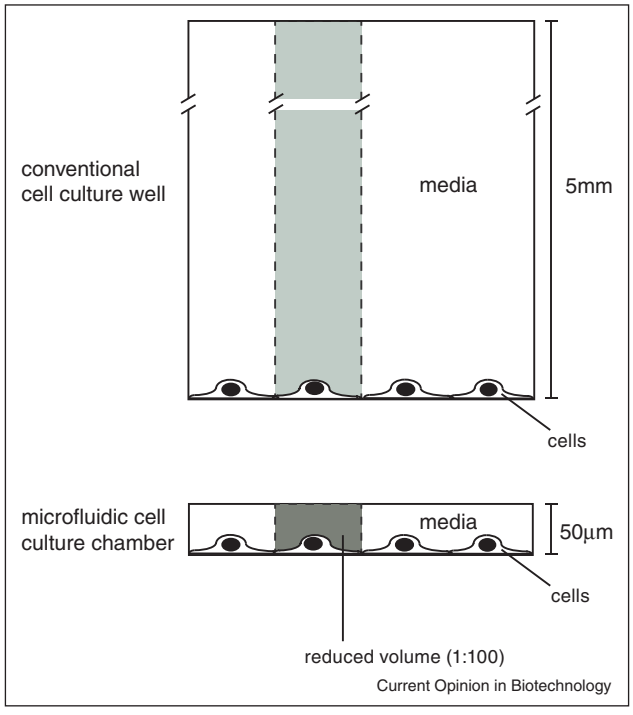


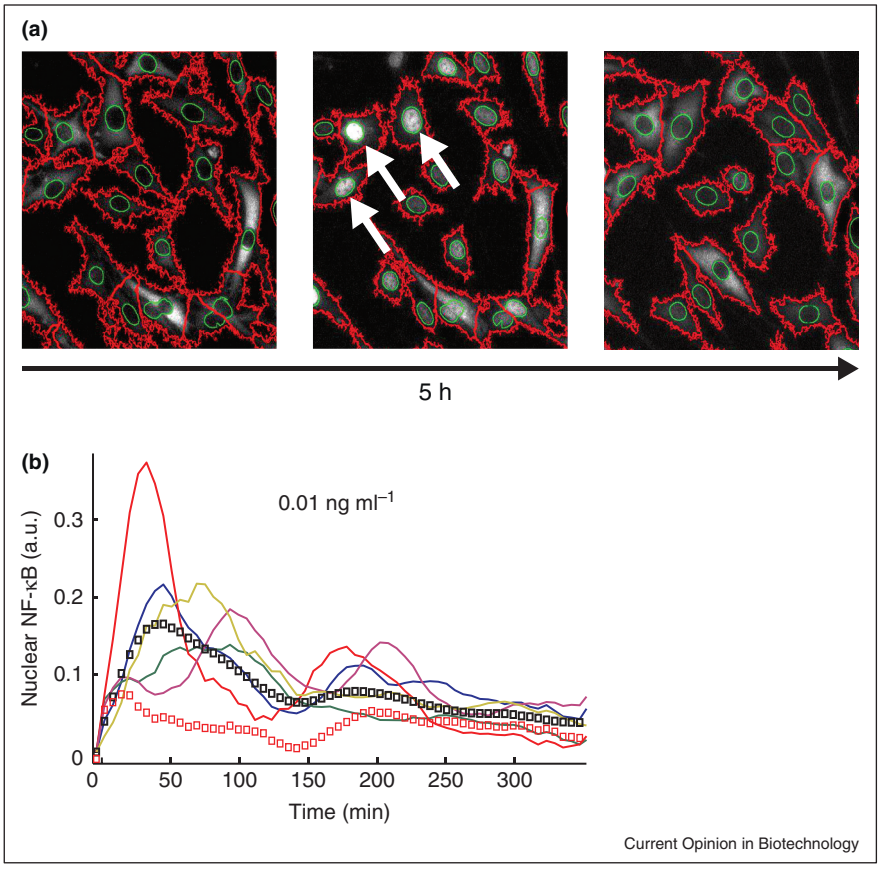
Illustration of '*small-volume effects*' in microfluidic cell culture devices. Smaller culture media volume for a given cell results in faster consumption of nutrients and increased concentration of metabolites or secreted molecules, similar to tightly packed tissues.

Recent developments in microfluidic cell culture systems and their applications

The numerous advantages of culturing cells in microfluidic devices resulted in a wide range of applications not only in classical biology, but also in systems biology, and biomedical and pharmacological research.

Systems biology has fundamentally transformed the understanding of biological functions on a cellular and organism level, and microfluidics continues to play an important role in this endeavor. The key requirements for systems biology in obtaining comprehensive datasets on a multitude of processes in parallel and over time while controlling specific environmental factors are readily met

Figure 3



Microfluidic cell culture devices allow precise dynamical quantification of biological processes at the single cell level. **(a)** Longitudinal real-time fluorescent images of fibroblasts expressing the fluorescent fusion protein p65–DsRed under control of the endogenous mouse p65-subunit of the NF-κB transcription complex (arrows illustrate activated cell nuclei). **(b)** Representative traces for NF–κB activity following stimulation with 0.01 ng/ml of tumor necrosis factor-alpha in single cells (solid lines), when only active cells are averaged (black squares), and when active and non-active cells are averaged together (red squares). Combining the traces of all cells shows a false picture of reduced activity. In contrast, single-cell traces reflect the correct digital nature and variable dynamics of NF–κB under low signal intensity.
Adapted by permission from Macmillan Publishers Ltd: Nature [24], copyright 2010.

by microfluidic cell culture. The use of microfluidics allows single cell level quantification of cellular responses to internal or external stimuli with a high temporal resolution (<1 s) over prolonged cell culture periods (Figure 3a). Parallelization allows the recording of millions of data points of given biological processes during a single experiment. A recent example is the characterization of the innate immune response of fibroblasts to the inflammatory cytokine tumor necrosis factor alpha (TNF-α) on a single cell level [24]. The use of high throughput microfluidic cell culture systems [16] allowed determining the effects of the magnitude, timing and duration of TNF-α exposure of fibroblasts on the activity of the transcription factor NF-κB. In contrast to population-level studies with bulk-assays, characterizing the activity of NF-κB in thousands of live cells by microfluidic cell culture and fluorescence time-lapse microscopy revealed that activation of cells is a digital process at the single cell level, subject to a great degree of variability or 'biological noise' (Figure 3b). Microfluidics further allow the integration of external feedback to control the expression of given genes over many cell generations [34]. In addition to capturing intracellular processes, microfluidics also provides quantification of extracellular responses to specific stimuli such as the production of cytokines from single cells [27••]. This allowed the quantification of temporal dynamics of the secretion of specific cytokines, revealing that T-cells produce cytokines asynchronously and in a sequential manner. Together these examples illustrate the potential of microfluidic cell culture systems to quantify biological processes at various levels, at a single cell level and with high temporal resolution.

Microfluidics also allows control of the cellular microenvironment, and does it in a highly parallelized fashion.

The concentration of biomolecules can be controlled spatially and temporally, thus allowing for instance the generation of diffusion-based gradients of given molecules [18•]. In these gradients chemotaxis in flow-free conditions (comparable to inflamed tissue) can be characterized. High-throughput microfluidic systems also allow detailed analysis of cell migration in a social context [17••]. As in previous examples, the quality and magnitude of the obtained data allowed compiling and calibrating highly accurate mathematical models for the observed biological processes. Further examples for the use of microfluidics in this field include the characterization of the variability of G-protein-coupled signaling pathways [35] and the bandwidth of the hyperosmolar signaling pathway [36].

High potential for parallelization in combination with long-term imaging qualify microfluidic cell culture techniques for capturing rare biological events. An example is the differentiation of stem cells [37•], which has been successfully achieved in microfluidic cell culture devices [38,39]. Controlling the spatiotemporal cues of the microenvironment and the ability to shape the geometry of cultured cells allowed studying primary neuronal cells and cell lines in microfluidic chips [40,41].

Generation of tissues *in vitro* for drug research and ultimately for therapeutic purposes was investigated using conventional cell culture techniques for many years. 3D scaffolds, though promising for achieving tissue-like connectivity, are quite limited in controlling the cell culture conditions, in nutrient and drug delivery, and in running simultaneous assays during cell culture [42]. Integration of microfluidics with such 3D scaffolding systems allows dynamic manipulation of culture conditions biochemically and biomechanically, such as creating dynamic 3D structures, and provides a microenvironment that allows formation of artificial tissues from cultured cells [43,44]. A recent example is the culture of blood vessel cells on the inner surface of micro-channels where flow and shear stresses can be controlled [45] and the generation of a vasculature system on PDMS-chips [46]. Other examples are renal and hepatic cells that have also been successfully cultured in close correspondence to the microarchitecture of the respective tissues [47,48]. In addition to these homotypic tissue culture models, heterotypic tissue culture models that mimic the respective tissue closely both from a histologic as well as from a physiological and functional standpoint have been achieved in microfluidic cell culture devices [49,50]. This allows high-throughput pharmacological studies and might ultimately result in using microfluidic cell culture systems also for regenerative purposes [51].

Outlook: future developments of microfluidic cell culture

The list of possible improvements to microfluidic cell culture is long, and we discuss some exemplary advances that will potentially broaden the use of microfluidics in the near future. Spatio-temporally controlled retrieval of single cells or confined subpopulations from microfluidic cell culture chips, perhaps through combination with optical or electromagnetic tweezers [52,53], would facilitate the seamless integration to other analytical methods commonly used in cell biology and diagnostics, such as high-throughput qPCR, mass spectrometry, and next generation sequencing. Improvements in the quantification of biological processes such as single cell gene-expression, RNA sequencing, or proteomic analysis of selected cells at given time points and further advancements in automation and feedback control [34,54] of microfluidic cell culture chips would considerably contribute to addressing fundamental biological questions in systems and quantitative biology. In addition to culturing and assessing individual cell types, further developments in coculture systems have the potential to widen the use of microfluidic cell culture devices, particularly in generating physiologically relevant *in vitro* scenarios of basic processes such as infection, innate immune response, and clonal expansion and selection of activated immune cells. The culture of intact tissues in microfluidics (i.e. a biopsy sample) for research and therapeutic purposes is possibly the next frontier in microfluidics research. Dynamic biochemical and biomechanical manipulation of cells and culture conditions has the potential to generate artificial tissues from dissociated cells [44].

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