Cell Systems

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Voices

How Can Microfluidic and Microfabrication Approaches Make Experiments More Physiologically Relevant?

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Microfabricated and microfluidic devices enable standardized handling, precise spatiotemporal manipulation of cells and liquids, and recapitulation of cellular environments, tissues, and organ-level biology. We asked researchers how these devices can make *in vitro* experiments more physiologically relevant.

Dissecting Biological Complexity

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The remarkable complexity that underlies human biology results from myriad interactions among different cell types and signaling proteins, yielding vast biological networks where spatiotemporal fluctuations between signaling nodes ultimately drive and tune biological systems. No matter the biological context—be it to understand the immune response to viruses, how tumors metastasize, or how stem cells respond to injury—it is clear that a simple Petri dish falls short in capturing and interrogating the intricacies of human biology.

A staple of the semiconductor industry, microfabrication has been widely adopted across biology and has introduced capabilities that biologists have long desired. At its simplest, microfabrication uses UV light to pattern arrays of high-resolution features across different length scales—from the microscale of single cells to the macro-scale of a bulk tissue—all in parallel and with high throughput. This control has enabled the engineered assembly of cells and proteins to create *in vitro* tissue models that more accurately recapitulate *in vivo* tissue microenvironments. Proteins can be patterned to mimic antagonizing signaling gradients during embryonic development, and cells can be patterned to capture specific tissue architectures and niches. Born out of microfabrication techniques, microfluidics can add "vasculature" to introduce important nutrients and soluble cues. Ultimately, microfabrication can catalyze new studies not previously possible, leading to advances in our understanding of crucial interactions underlying human biology and disease.

Improve Reproducibility!

Petra Schwille
Max Planck Institute of Biochemistry

The obvious answer to this question is that microfluidics, and microsystems technology in general, can greatly help to downsize and standardize all kinds of *ex vivo* experiments, which is beneficial in many ways. But I wonder whether it is particularly the "physiological relevance" that is improved by microfluidics. Even more, I am unsure whether that is such a useful criterion altogether. What does it mean when an experiment is physiologically relevant? That it will help to fight diseases? Or that we actually understand biology better? As a physicist working in biology, I have found physiological relevance to be my natural enemy, because as soon as you believe to have understood a phenomenon, and a reviewer asks for more experiments to make it more physiologically relevant, you can be pretty sure that you don't understand it any more afterward. Therefore, I have convinced myself that in order to make statements about biological systems that have a longer shelf life than a couple years, you'd better sacrifice physiological relevance for simplicity and create well-defined and reproducible assays in which you work with purified components or extracts whose composition you can reasonably well control. My personal dream is to create a minimal cell bottom-up from a defined set of functional components. Here, microsystems technology is immensely powerful for standardized handling, but also to create 3D microenvironments that mimic the cellular habitat in scale and shape for protein systems to unfold their functionality.

Enabling Physiological Conditions

Andreas Hierlemann
ETH Zürich

Microtechnology and microfabrication approaches have fueled research in biology in the last years. With features and dimensions on the length scale of single cells, microfluidic devices enable precise spatiotemporal control and manipulation of cellular microenvironments *in vitro*. Parameters, such as temperature, compound concentrations, flow rates, or mechanical forces, can be adjusted to reproduce—as closely as possible—*in vivo* physiological conditions.

Liquid flow can be continuous or pulsatile, as it is in different biological environments, and mechanical or biochemical cues can be tailored to mimic specific cell environments, for example, niches in which stem cells form or differentiate. Cell-cell interactions that naturally occur in tissues can be realized by embedding cells in hydrogels or by replacing traditional 2D cell cultures with 3D microtissues, which is expected to yield more representative results in *in-vitro* drug testing. Even tissue-tissue interactions can be realized by fluidically interconnecting, e.g., cancer and liver microtissues, to recapitulate metabolic transformation effects or to mimic liver-mediated activation of prodrugs, which greatly affect drug efficacy and/or toxicity. Moreover, microfluidic techniques can be used to reproduce physiological compound-concentration profiles in a human body upon oral or intravenous medication uptake.

Finally, microtechnology also includes microsensors and microelectronics, which offer the possibility to interact with cells or tissues that react to or produce electrical signals, most prominently heart or brain.

Controlling Space and Time

Savas Tay
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Biological systems are inherently complex and dynamic, and experiments need to capture these features to be relevant. Tissues consist of many molecules and cell types whose numbers, positions and configurations must be determined to understand and model their functions. Moreover, these properties constantly change. Such dynamics is central to processes like metabolism, immunity, development, and to many diseases. If we can generate accurate and reproducible datasets with sufficient complexity and spatiotemporal resolution, we can build computer models that will lead to a revolution in basic biology and medicine.

Automated microfluidics can enable this revolution. Microfluidics allows controlling the temporal and spatial scale of experiments precisely in a very broad range, from micrometers to centimeters, and from milliseconds to days. Using microfluidics, cells can be pre-arranged in 2D and 3D co-cultures with predefined positions to mimic tissues and be exposed to dynamic chemical stimuli that mimic natural signaling systems. Dose, timing, duration, combinations and order of signaling molecules can be precisely controlled using microfluidics.

Microfluidics improves the accuracy and reproducibility of biological measurements by reducing pipetting errors. Moreover, parallelized microfluidic systems greatly increase the throughput of biological experiments. Finally, combination of automated microfluidics with closed-loop feedback control and artificial intelligence can reveal truly autonomous experimental systems that can drive (or evolve) biological systems to a desired outcome without human intervention.

Organs-on-Chips

Josep Samitier
University of Barcelona

Today, its envisioned that having complete control of the flows at micrometric scales combined with cell cultures will allow the construction of systems that can mimic complex physiological functions. The concept of "organ on a chip device" (OoC) that enhances "life-like" environments for cell culture, aims at a better approximation of *in vivo* cellular organization, function, and interactions than simple 2D tissue-culture systems. OoC attempts to reliably reproduce the biological and physiological *in vivo* characteristics, including different cell co-culture to reduce the use of animals. It is an interesting alternative due to its versatility, controlled rapid analysis, repeatability and low-cost design to mimic both *in vivo* physiological and pathological conditions.

Would OoC be able to mimic organ-level functions? The challenges facing the development of living systems on chips are multiplied by the fact that each organ represents its own set of challenges. The small sample volumes involved makes collecting and analyzing metabolites difficult, the effects of polymers and microfluidics on cell behavior are still poorly understood, and integration of multiple OoCs is in the frontier of the present technological capabilities. Probably the evolution of dynamic control of different media and cues for cell culture, the *in situ* on-chip real-time visualization of molecular processes, the improvements of the 3D printing (and bio-printing) technologies and the use of cells from patients for personalized disease modeling would be key issues for OoC development.

Multicellular Microfluidics

Jianping Fu
University of Michigan

Microengineering tools have been developed to provide new functionalities for dynamic quantitative measurements and perturbations down to the single-cell level. These tools have also become more widely adopted by biologists to tackle their favorite questions. While this trend will continue, given the need to maximize the physiological relevance of *in vitro* culture systems, however, the complexity and dynamic nature of physiological environments cannot be understated.

Microengineering tools are successful in studying cell migration and tumor angiogenesis and establishing human "organs-on-chips." Importantly, there are exciting emerging areas where they will make important impacts, such as multicellular development and self-organization and developmental bioengineering. Microengineering tools are powerful for controlling initial seeding and positioning of single cells and cell clusters. Furthermore, microfluidics can provide local signaling centers/sources to trigger symmetry breaking and establish dynamic signaling gradients, critical for pattern formation and tissue morphogenesis. Microengineering tools can also allow integrated controls of both soluble biochemical signals and insoluble biophysical cues and studying the roles of tissue geometry and mechanical forces in influencing cell signaling and cell-cell communication in multicellular development. Another critical aspect for microengineering tools to address is scaling up to ensure their compatibility with prolonged culture and continuous growth of multicellular structures while maintaining their unique advantages.

Beyond Just Shear Forces

Peter Loskill
University of Tübingen

Combining microfabrication and tissue engineering not only leads to an unprecedented control of structural, biochemical and mechanical parameters of the microenvironment but also enables the creation of microfluidic channel architectures, a key aspect of Organ-on-Chip technology. These channels feature similar dimensions as the human microvasculature and allow for a perfusion of tissue constructs with "blood surrogates." Often-discussed facets of perfusion are shear forces and their effect on cell morphology and functionality. However, the impact of microfluidic perfusion on the physiological relevance goes far beyond this aspect as it, e.g., allows for physiological cell-to-media ratios and vasculature-like transportation processes. Physiological cell-to-media ratios ensure that cells are at any time in contact only with a small media volume preventing dilution of secreted factors important for autocrine and paracrine signaling. Vasculature-like transportation processes enable the generation of precisely controllable (stable) conditions, a continuous delivery of fresh nutrients at physiological levels, and a removal of metabolic products. In contrast, in conventional static culture, a significant portion of nutrients is consumed already after two days, depending on the metabolic activity of the cells. At the same time, there is a continuous buildup of secreted factors and metabolic (waste) products. This means that culture conditions vary continuously, initial nutrient levels have to be at non-physiological high levels and periodic media exchanges create non-physiological rhythms and artificial dynamics.