



Bench and Bedside - Bench

## Organ-on-a-chip and the kidney

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Traditional approaches to pathophysiology are advancing but still have many limitations that arise from real biologic systems and their associated physiological phenomena being too complicated. Microfluidics is a novel technology in the field of engineering, which provides new options that may overcome these hurdles. Microfluidics handles small volumes of fluids and may apply to various applications such as DNA analysis chips, other lab-on-a-chip analyses, micropropulsion, and microthermal technologies. Among them, organ-on-a-chip applications allow the fabrication of minimal functional units of a single organ or multiple organs. Relevant to the field of nephrology, renal tubular cells have been integrated with microfluidic devices for making kidneys-on-a-chip. Although still early in development, kidneys-on-a-chip are showing potential to provide a better understanding of the kidney to replace some traditional animal and human studies, particularly as more cell types are incorporated toward the development of a complete glomeruli-on-a-chip.

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## Introduction

Technologic advances change our lifestyle in many ways. New electronic devices become ever smaller but with ever increasing functions, so they can replace large space-occupying equipment. A few decades ago, microfluidics was developed using advances in the semiconductor industry and the subsequent expansion of microelectromechanical system field [1]. The technology can manipulate at the microscale and control fluid flow precisely. Microfluidics has provided new capabilities to researchers in biology and medical science. Since early 2000, published articles and patents on microfluidics have increased dramatically in the field of engineering. Importantly, although still a smaller overall fraction, articles on

the use of microfluidics in the medical field are growing most rapidly at an exponential rate in recent years [2].

Microfluidics is characterized by the engineered manipulation of fluids at the submillimeter scale [2]. The microscale devices in microfluidics are commonly referred to as miniaturized total analysis systems or lab-on-a-chip technologies [2]. In the 1990s to 2000s, microfluidics focused on the miniaturization of previously macroscale assays. Within several years, various new technologies showed better performances than the traditional methods. For example, there are paper-based analytical devices for diagnostics [3], immunoassays assisted by surface tension [4], and organ-on-a-chip technologies [2,5] (Fig. 1). We will further focus on organs-on-chips.

## Organ-on-a-chip

Organ-on-a-chip is a microfluidic cell culture device that is a more physiologically relevant *in vitro* model than cells cultured in dishes. Important features include continuously

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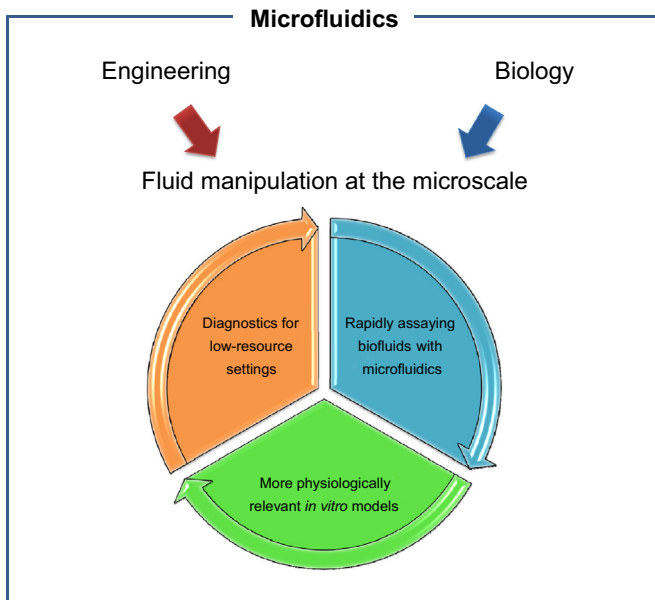


Figure 1. Concepts of microfluidics.

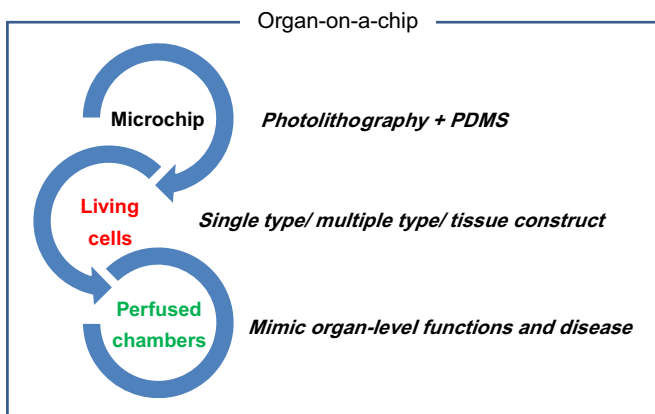


Figure 2. Concepts of organs-on-a-chip. PDMS, polydimethylsiloxane.

perfused chambers inhabited by living cells arranged to simulate tissue- and organ-level physiology [5] (Fig. 2). So far, several individual organ-on-chips have been developed, including gut-on-a-chip [6], lung-on-a-chip [7], blood vessel-on-a-chip [8], cancer-on-a-chip [9], bone marrow-on-a-chip [10], and kidney-on-a-chip [11]. In addition, multiple organs-on-a-chip has been tried, such as liver-tumor-bone marrow-on-a-chip [12] or liver-skin-intestine-kidney-on-a-chip [13].

As shown in Fig. 3, the whole experimental process using organ-on-a-chip technology consists of several steps. Photolithography, a fabricating technique that can make channels in a microscale, comes from semiconductor technology. It requires labor-intensive work, clean environments, and sophisticated training. An organ-specific microenvironment is a basic platform in the design of these devices. Briefly, researchers design the optimal condition with the proper size, numbers, and compartments of the channels. According to these designs, fine molds are cultivated and the final devices can be made out of transparent and soft polydimethylsiloxane (PDMS) [5]. Sometimes, simple ways to make replicas can be

used such as xerographic printing or three-dimensional (3D) printing of molds.

Cell sourcing is an important issue in the organs-on-a-chip field. Usually, a single cell type may be enough to mimic minimal organ function. For example, a kidney-on-a-chip for the evaluation of direct cellular toxicity may be not enough when constructed with renal tubular cells alone [11,14]. To mimic more physiologic conditions or evaluate cell-to-cell interaction, two cell types should be used like lung-on-a-chip [7], which has cultured endothelial cells on the bottom channels and alveolar cells on the top channels. This lung-on-a-chip also introduced human neutrophils with culture media to the bottom channels to observe the movement of inflammatory cells. Sometimes, cells in a tissue construct itself may be introduced into the devices, such as skin tissue or intestine constructs [13]. Humans-on-a-chip has multiple compartments to allow evaluation of the interaction among interesting organs [13].

#### Why is organ-on-a-chip a hot topic?

When new drugs are developed, drugs should be tested in three different stages: preclinical, clinical, and postapproval stages. Preclinical studies include two kinds of experiments: cell culture and animal studies. Cell culture methods are just culturing living cells on a dish; there is no flow of blood, and the conditions are different from cells in a real organ. In addition, the data from animal studies are not always predictive of human responses. They have fundamental limitations such as different physiology and different cellular functions. Kidney toxicity is one major cause of drug attrition and failure. Only 2% of drug development failures are screened in the preclinical stage, and serious adverse effects in > 20% of new medicines are discovered only after the clinical stages [15]. The reason why preclinical studies cannot better predict kidney toxicity is due to the limitations of current preclinical cell culture and animal models.

We need real human organ-like devices that are superior to animal models. Organ-on-a-chip may be a good solution, which has minimal functional units that use primary human cells, rather than animal cells, like a real human organ. The ideal methods will not only use human cells but also mimic 3D architecture and flow conditions within real human organs. Microfluidic devices seeded with human cells and perfused with cell culture media in a physiologically relevant manner have already been developed to provide a minimal functional unit to mimic real organs. The small size allows easy flow control and requires few cells and only small volumes of samples and reagents. Parallel experiments with large numbers of samples at the same time can also be realized. An additional advantage of the devices is optical transparency that allows visualization, at the cellular level, of the whole drug response process, something that is difficult to do in actual living organs.

#### What are the pros and cons of organs-on-chips?

Raw materials used in these systems are inexpensive, but specialized microengineering capabilities are needed and some, such as cleanrooms or pumps, can be expensive. Other challenges, such as bubbles, arise because of the small size. Before or during the perfusion of fluid, bubbles can form easily in the channels of devices but are difficult to remove. Cells in the device are very susceptible to detachment and injury from

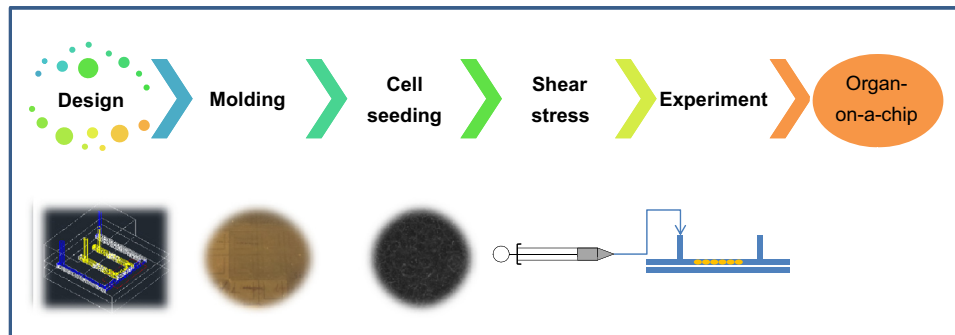


Figure 3. Simple example of an experiment using organ-on-a-chip.

such bubbles, although in some cases, such air-liquid interface-mediated injury itself can be physiologically relevant such as mimic lung injury [16]. In most cases, researchers should carefully avoid bubbles or dust. For attachment of the cells on the membranes, extracellular matrix coating is necessary. As culturing time increases, the matrix becomes degraded gradually, which can affect cell viability. It is also challenging to achieve consistent cell seeding and prevent contamination of microbes as many experiments require prolonged cultures of several weeks. Organ-on-a-chip systems are still not suitable for certain areas such as chronic diseases modeling, adaptive immune responses, or complex system-level behaviors of the endocrine, skeletal, or nervous systems [5]. To date, about 4 weeks may be the longest perfused models of organs-on-a-chip [13]. Culturing suspending cells in organ-on-a-chip is still challenging, although introduction of various types of attaching cells in a chip is relatively easy [13].

In contrast, smaller experimental samples and scaling up to larger numbers of samples can be advantageous. Through fluid flow control, researchers may enhance differentiation, function, and viability or regulate microenvironmental signals. Prediction of pharmacokinetic and pharmacodynamic properties of drugs may benefit from the ability to adjust renal clearance rates and to incorporate metabolism of an interconnected liver compartment. Depending on the design of the synthetic culture system, many different parameters can be controlled, such as types and positions of cells; orientation of tissue-tissue interfaces; transcellular chemical, molecular, and oxygen gradients; flow levels and patterns; and mechanical forcing regimens. A 3D cell culture can be more accurate models of *in vivo* cells than 2D cell culture, but 3D culture methods have been evaluated under the static condition instead of dynamic conditions so far. Some combined models between organ-on-a-chip and 3D cell culture could be an optimal condition for mimicking *in vivo* microenvironments.

### Kidney-on-a-chip

The initial design of a published kidney-on-a-chip has two compartments [11,14]. A top channel mimics the urinary lumen and has fluid flow, whereas the bottom chamber mimics interstitial space and is filled with media. Kidney cells are under much lower shear stress than the endothelial or lung cells. This device used rat distal tubular cells or MDCK cells, and its shear stress was  $\sim 1$  dyn/cm<sup>2</sup> [11]. A second report showed the same design, but human proximal tubular cells were used. In this model, the authors tried to reproduce cisplatin nephrotoxicity. Proximal tubular cells have much lower shear stress  $\sim 0.2$  dyn/cm<sup>2</sup> [14].



Figure 4. Application of kidney-on-a-chip.

The foot processes of podocyte, a glomerular visceral epithelial cell, form a size- and charge-selective barrier to plasma protein, and derangement of the barrier causes podocyte injury and proteinuria [17]. Some scientists have tried podocyte-on-a-chip, but it is still challenging. It may be because podocyte is exposed under a very low shear stress *in vivo* and requires a sophisticated culturing condition [18].

Kidney-on-a-chip has various potential applications (Fig. 4). Cisplatin nephrotoxicity was evaluated in the kidney-on-a-chip using human proximal tubular cells [14]. Cisplatin was introduced into the bottom space, and cisplatin-induced cellular damage was monitored to the cells for 24 hours. During the following 72 hours, shear stress was helpful for facilitating recovery of the injured cells and associated biomarkers. Shear stress in the devices can facilitate translocation of aquaporin-2 and relocation of actin cytoskeleton in the kidney-on-a-chip using primary cultured inner medullary collecting duct cells of rat kidneys [19], which is a good example of a physiological experiment using kidney-on-a-chip. Moreover, renal tubular epithelial cells are continuously exposed to the changes of extracellular microenvironment, e.g., transepithelial osmotic gradient, and changes of luminal or interstitial pH. The effects of these physiological factors on the functions of renal tubular cells could also be investigated by exploiting microfluidics [20,21]. If the offending

antigens or mechanisms of the diseases are well identified, disease modeling can be easily fabricated in the kidney-on-a-chip. Kidney injury models using nephrotoxic drugs may be simple candidates for the application. Kidney-on-a-chip is only one compartment of an eventual multiorgans-on-a-chip system [13]. To research drug metabolism, renal excretion or metabolism should not be omitted. However, some multiorgans-on-a-chip systems have been using kidney tubular cells not for evaluating renal excretion, but for measuring kidney injury. Animal renal clearance is usually higher than human renal clearance. Using pharmacokinetic data from animal models runs the risk of underestimating human nephrotoxicity. Applications of human kidney-on-a-chip may fill such gaps through control of drug concentrations and flow rates that mimic human drug clearance or metabolism.

### Directions for future research

PDMS can absorb small organic compounds. Lipid soluble compounds, such as hydrophobic drugs, are incompatible with PDMS devices because of absorption into PDMS. The same extracellular matrix coated on PDMS can have different effects when coated on different surfaces such as glass or polystyrene or in the body in a cell type-dependent manner, requiring much trial and error. There is potential need for a universal blood substitute to perfuse multiple organ compartments. Some studies used a mixture of various cell culture media. Other studies used different culture media for each compartment. Another issue is scaling. Isometric shrinkage of organs creates an unbalanced system between organs because of functions that depend on organ volume versus organ area. There are multiple ways to scale down each organ, and what is most appropriate is still controversial [22,23]. Automated control systems can be helpful and may allow nonspecialists or academic researchers to replace 2D or 3D culture systems. Some companies have already launched commercial use of organs-on-a-chip. Pharmaceutical and biotechnology industries have a significant need for validation and prioritization of lead drug candidates. In addition, clinical trials may be able to adopt new biomarkers of drug efficacy, toxicity, or disease response developed using these devices in the near future.

Microfluidics is still a young and developing field. However, there are many opportunities for biologists and medical scientists to collaborate with engineers. For example, 3D organoid cultures, dynamic cultures, and disease modeling are areas where collaborations have led to better understanding of pathophysiology and mechanisms. Multiorgan-on-a-chip or human-on-a-chip is a young but exciting area. In the nephrology field, glomeruli-on-a-chip, kidney organoid, and nephron-on-a-chip are still challenging. Organs in the human body are vital but difficult to manipulate and image at the cellular level, making evaluation of their functions and responses difficult. In contrast, the use of organs-on-a-chip is relatively cheap, readily imaged, and easily accessible for measurement of functions and will provide a vital window into human physiology in ways not currently possible.

### Conflicts of interest

The authors have no conflict of interest.

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