

# Development and Applications of Microfluidic Devices for Cell Culture in Cell Biology

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## Abstract

Development of microfluidic culture technology combined with tissue engineering catalyzes the progress in study of cell biology. These tools will promote the understanding of physiological and pathological changes. Cancer cells and stem cells are sensitive to their surroundings, thus could be better explored by controllable microfluidic devices. In this review, we describe the ways to control cell microenvironment and explain how the influencing factors influence cellular behaviors, then present microfluidic-chip-based exemplary applications for cancer models and stem cell differentiation.

**Keywords:** 3D cancer model; Cell patterning; Microfluidic culture; Cell biology; Stem cell differentiation

## Introduction

There is a clear need to study normal and pathological cell function under native *in vivo* milieu. As many of problems of interest in cell biology lie on micro scale, there is an urgent need to process studies at micro level. The miniaturization of culture system fulfills this requirement due to its features like automatic operation, high-throughput analysis highly integration of function modules and precisely control of different parameters. Among these microfluidic culture systems, 3D microfluidic culture models are powerful to improve the physiological relevance of *in vitro* models in study of cancer [1,2]. In addition, the development of *in vitro* models at tissue and organ level is one of the most promising microscale applications in high-throughput screening of drug toxicity. The integration of tissue-engineering strategies and microfluidic technologies has recently sparked a breakthrough in adapting to morphological changes in tissue structure and function over time, providing a level of precision control that could not be achieved previously [3]. Tissues-on-a-chip and organs-on-a-chip show a more humanized character, would greatly improve the efficacy of drug toxicity screening [4,5]. Vascular structures have been constructed to explore inflammatory signals and further to connect individual organs to form a microvascular 3D networks [6]. There are a number of journal papers that are published in microfluidic culture applications in life science and some microscale 3D *in vitro* systems are commercially available for applications of anti-cancer drug testing [7], reflecting the growing importance of this field.

Based on our previous work, we review the development of microfluidic applications in cell biology in three sections. In the first section, how the cells and microenvironment factors are patterned and controlled to replicate *vivo*-like models in microfluidic devices are described and how the factors influence cell fate are discussed in terms of physical cues, cell-cell and cell-ECM interactions. The second section introduces the cell-assays based on the development of 3D microfluidic culture in the field of cancer research and stem cell differentiation. Finally, we describe potential future of microfluidic culture in cell-biology study.

## Cell and environment control

Cell fate *in vivo* is largely affected by external factors like physical or chemical interface and interaction with other cells or matrix; therefore, it is important to precisely control these factors in *in-vitro* studies. Microfluidic cell cultures wins over conventional culture methods in controlling these factors both spatially and temporally, and then shedding light on mechanisms of cellular processes *in vitro*.

**Cell patterning:** Adhesion is a fundamental behavior that determines cellular behaviors like polarity, migration and apoptosis [11]. In others words, the impact of geometry by a cell and a group of cells could be studied by controlling the adhesion behavior. The adherent state of cells on surface can be governed by chemical and mechanical methods in microfluidic devices (Figure 1a). For example, Dertinger's group processed neural network construction by using surface gradients of laminin, an ECM protein important for neuronal guidance [12]. They found anon orientation toward the increasing surface density of laminin. Using a piece of hydrophobic polythyleneterephthalate (PET) film, we separated two kinds of cells on the same coverslip before the following on-line analysis of metabolism [13].

On the contrary, topographical factor shows no significant impact on cell patterning. By creating continuous sinusoidal features in PDMS, cells grown on this surface were proven to show similar alignment on completely smooth wavy and sharp corners [14].

Micro compartmentalization has enabled patterning cells *in vivo*-like cellular arrangements by channel geometry and the physical attributes of microsystems. For example, our group investigated the quantum dot (QD) cytotoxicity on HepG2 cells cultured in agarose matrix. The 3D culture chambers were divided into close and far chambers for their different distances from the main channel [15].

Zervantonakis's group regenerated endothelial barrier in 3D microfluidic model [16]. The 3D compartmentalization enables precise investigation of distance between tumor and stromal cells, and the incorporation of macrophages (the cells that direct tumor intravasation). The distance dependence has also been studied in terms of breast cancer progression [17]. Cancer cells that in closer proximity to fibroblasts were proven to be more invasive. Therefore, in the process of cancer progression, the physical contact with fibroblasts might be the basis before the driving by soluble factors.

Fabricating microwell array on microfluidic platform is a typical

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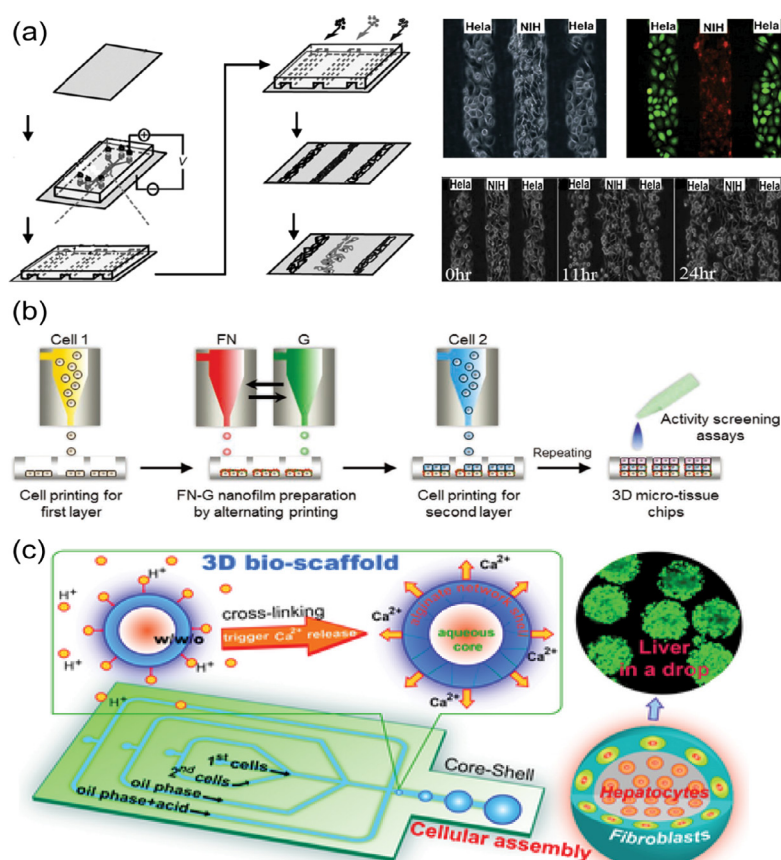
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method for cell patterning. We have made several researches by this method to control cell density gradient and for single-cell analysis [18-20].

To confine groups of cells into designed geometric shape, another way that has risen up in the recent years printing by inkjet, especially for 3D model construction. Taking advantages of automatic, high-throughput and high-efficient operating of solution at micro/nano level by this method, our group have made successful progress in single cell analysis and cell patterning [21,22]. This approach allows the assembly of heterogeneous tissue structure to mimic *in vivo* physiological tumor model, as well as the possibility of fabricating 3D *in vitro* tumor models with large-scale, high throughput and high cell density. It is promising to produce reliable *in vitro* models from 2D to 3D. Based on the rapid and automatic inkjet technology, different cells could be deposit in predefined patterns [9,23] (Figure 1b). To construct 3D co-culture, cells can be mixed with collagen by a dual ejection [24]. Successful applications of this method were seen in exploration of breast cancer initiation and progression [25]. The 3D tumor assembly can be controlled by precisely mixing of cells and matrix substrate like gelatin and alginate, thus shows advantages in mimicking *vivo*-like micro environmental characteristics. Similar examples of application of cell printing had been reported in controlling the stem cells. Dolatshahi-Pirouz [26] reported high-throughput generation of miniaturized and combinatorial cell-laden micro gel arrays for screen of various biomaterials in combination with selected soluble factors for MSC osteogenic inductive.

As previously mentioned [27], scaffold-based culture model is advantageous for simulating the *in-vivo* microenvironment and provide better mass transport efficiency than natural-derived materials. This structure benefits from biomaterials that support well-control of cell patterning. With the aid of computer, 3D porous bioactive scaffolds with complex architectural structures and well-defined material properties could be rapidly fabricated [28,29]. The second way to fabricate 3D scaffolds is electrospinning, which creates non-woven mats volume as ECM analogue scaffolds. There are two major advantages of this method: one is the cost efficiency of using very small quantities of polymers, and the other is that additional components such as co-polymers and growth factors can be added to the polymer solution in the preparation process and then be incorporated into the electrospun fibers. The scaffold based models are suitable for investigation of anti-cancer drug mechanisms. In a recent work, a core-shell scaffold had been used to spatially assemble hepatocytes in the core and fibroblasts in the shell, which realized cell-cell interaction in a drop (Figure 1c).

**Control of physical factors:** Microfluidic systems can help elucidate the physical factors (such as oxygen, shear stress, geometry and temperature) that affect the behavior of cells. We had investigated the impact of oxygen on migration of cancer cells on an integrated microfluidic device [30]. The Caski cells showed slower migration rate under 15% of oxygen than that under 5% oxygen. The impact of shear stress is mainly discussed on vascularized tissues or mammary ducts. For example, fluid shear stress was indicated to mediate endothelial cell transcription, proliferation, barrier function, and changes in



**Figure 1:** Cell patterning forms. (a) Cells patterning via electrochemical control [8]. (b) 3D micro-tissue arrays by printing of single cells and proteins [9]. (c) Controlled assembly of heterotypic cells in a core-shell scaffold [10].

actin cytoskeleton rearrangement. Various microfluidic devices have been developed to control the shear stress applied on cells. The two-independent-channel types, which separated by a gel/scaffold compartment, has been used often to control [31] or study the influence of shear stress on cultured cells. The microfluidics shows advantages for allowing the flow to be controlled precisely in the channels as well as across the gel compartment [32,33]. Both micro environmental components and intricate architecture of the micro-vasculature networks strongly influence the fluid shear stress.

The influence of shear stress can be limited by separating the cells from the flow with a barrier, such as hydrogels [27-29], nano porous membranes [30], and micro channels [28], that allow passive diffusion of biomolecules.

Temperature also affects cellular behavior. To study the impact of temperature on microfluidic systems, perfuse temperature-controlled liquid were introduced into the systems. The control of temperature is realized by two distinct laminar-flow-based systems [38,39]. This approach has been applied on revealing the dependence of temperature for development rate of embryo [38]. Similar successful application was achieved activating or deactivating temperature sensitive genes in cytoskeletal dynamics study [33].

We have reviewed the types of microfluidic culture in previous work [27]. And due to the spatial constraints of micro channels, two-dimension (2D) culture show dominance of diffusion over convection. This feature of mass transport also controls the transport of signaling molecules in 3D micro systems but is governed mostly through the external cellular matrix (ECM).

The *in vivo* microenvironment is a confined space for cells to live. The confinement has impact on cell structure and behavior. Compared to single channel, micro-compartmentalization devices are more capable to rebuild this confinement, especially for physical confinement encountered by cells during migration and invasion, creating *in vivo*-like cellular arrangements [17,41].

Traditional cell migration or invasion studies are conducted in a vertical device, which hinders the real-time monitoring. However, microfluidic devices allows horizontal arrangement of compartments for better monitoring of changes in cells and the ECM during migration, therefore are more, suitable for analysis on dynamic cell transition. There are several successful reports about cell motility, for example, study the regulation of dendritic cell migration [42]. Researchers have found that mechanical confinement itself has impact on cell motility [43].

**Gradient of chemical factors:** Chemical concentration gradients are regulated to control many basic cell functions and biological processes such as gene regulation (MAPK-mediated bimodal gene expression and adaptive gradient sensing in yeast), cancer metastasis [44,45] cellular chemotaxis [37,46] and migration [47,48], differentiation, development [49,50], immune response [51,52], wound healing [53,54] and embryogenesis [55].

Early *in vitro* platforms to study the effect of chemical gradient, like Boyden chamber [50], Dunn slide chamber [57], Zigmond chamber [58] and agarose/petri dish [59], are limited to reach the length scales that are actually relevant to biological cells. Microfluidic device overcomes these shortages and offers higher gradient resolutions and provides well-controlled hydrodynamic and mass transport conditions. The temporal and spatial control over defined gradients of soluble factors or immobilized factors (on surfaces) provided by flow-based microfluidic devices is a significant improvement over the widely

available methods. The effect of flow alone on neutrophils has been addressed and mechanical activation by shear from laminar flow in micro channels was demonstrated [60].

**Cell-cell and cell-extracellular matrix (ECM) interactions:** Cell-cell interaction is realized by cell-cell junctions or paracrine signaling mechanisms, and determines the response to stimuli and cellular phenotypes. Microfluidics offers opportunity to isolate specific signals at single cell level and obtain a clear understanding of genotypic and phenotypic variation among similar cell type by high-throughput screening. By directing interactions between cell surfaces, specific homotypic or heterotypic cell-cell interactions can be studied. [61,62].

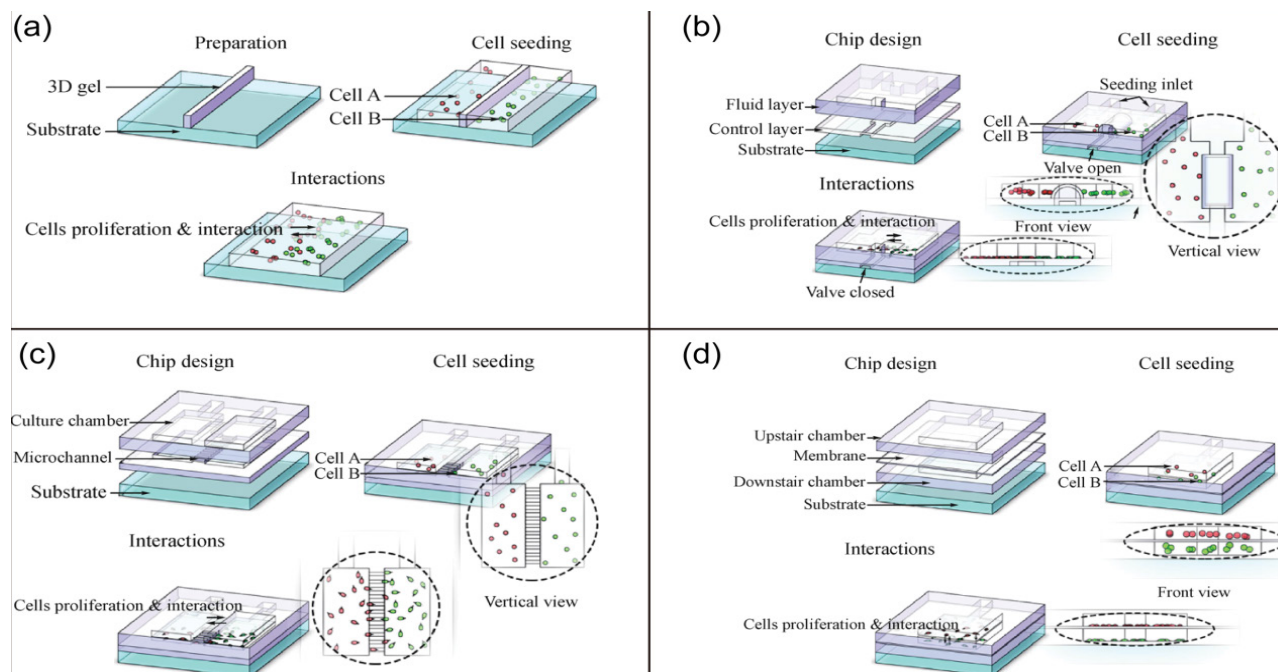
Generally, seeding of different types of cells is the first step for cell-cell interaction study. Unlike 2D culture, 3D culture model typically needs to premix cells with matrix (Figure 2a). The 3D structure provides the foundation of human complex tissue network and is proper to serve as a study model in microfluidics. Other designs of chips for cell-cell interactions include microvalves, microchannels and membrane as shown in reference [63-68] (Figure 2b-d) and to control administration of metabolites from one kind of cells to the receptor cells, our group had once presented a so called surface tension plug on a microfluidic device. Based on this device, we investigated the signal pathway between 293 and L-02 cells [69]. Paracrine communication is usually studied on a two-channel type that separated by a gel channel or intervening gel. In this model, interstitial flow application induced 3D tissue-like structures, which proved the enhancement of cell-cell cohesion. [70].

Li's group used electrochemical desorption of self-assembled monolayers (SAMs) to control multiple types of adherent cells in their migration behaviors in real time [8]. The first electrochemical desorption of SAMs in fibronectin filled PDMS channels induces the adhesion of cells in the channels. After the PDMS stamp was peeled off, a separation of different types of cells without physical barriers was formed. Then a second step is desorption releases the remaining SAMs and enables cells to spread freely.

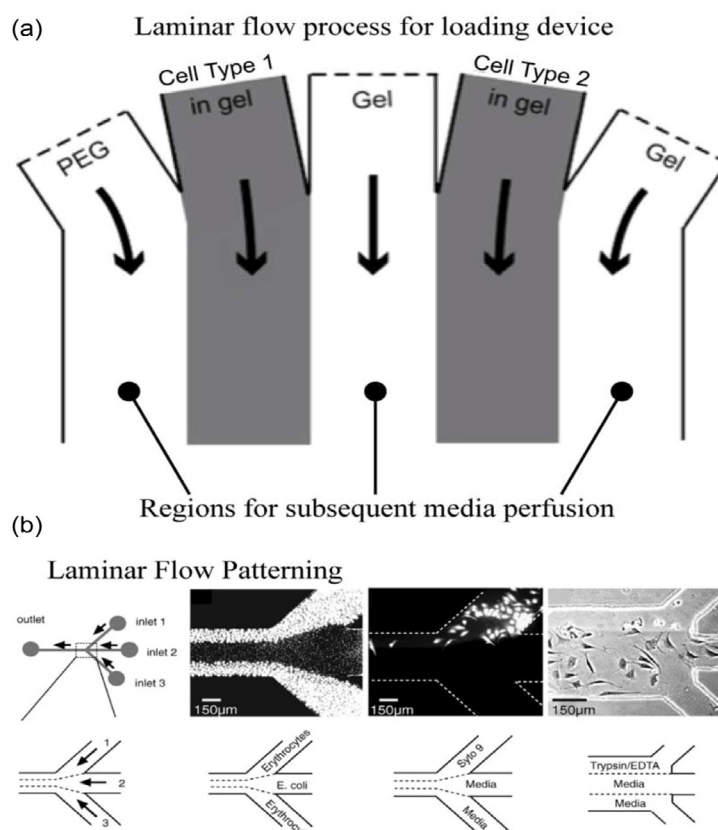
The composition and physical features of ECM directly influence a range of cellular processes including cell life/death, differentiation, shape, polarization, and motility [71-75]. Therefore, the ECM surrounding cells is critical to determine the cell fate. Various ECM materials have been used including naturally-derived polymers (collagen, hyaluronan and fibrin) and artificial polymers (alginate, polyethylene glycol (PEG), and poly (lactic-co-glycolic) acid (PLGA)). Naturally-derived ECM materials are biocompatible but limited in cost and repeatability, while artificial polymers are readily controllable in architecture, stiffness, porosity and shape. Fischbach's group had reported the use of arginylglycylaspartic acid (RGD)-peptide that incorporated into a 3D alginate ECM, which increased angiogenic activity of cancer cells [76].

Laminar-flow microfluidics is an attractive approach to generate a linear gradient using small amounts of molecules, and thus is suitable for conducting studies on specific cell-ECM interactions (Figure 3) [12,78]. For example, Dertinger showed microfluidic application on haptotaxis study based on a T-sensor network, and analysis showed that the surface density of laminin oriented axon specification. Hsu used a parallel flow chamber to study the influence of shear stress and investigate the interactions between haptotaxis and shear stress during EC migration. In 3D microfluidic vessel model, luminal combined with interstitial flow are major factors to regulate cell-cell and cell-ECM signaling. The interstitial flow is the extracellular fluid that exists in the interstitial spaces between tissue spaces, can direct the migration of cancer cells [79] (Figure 4).

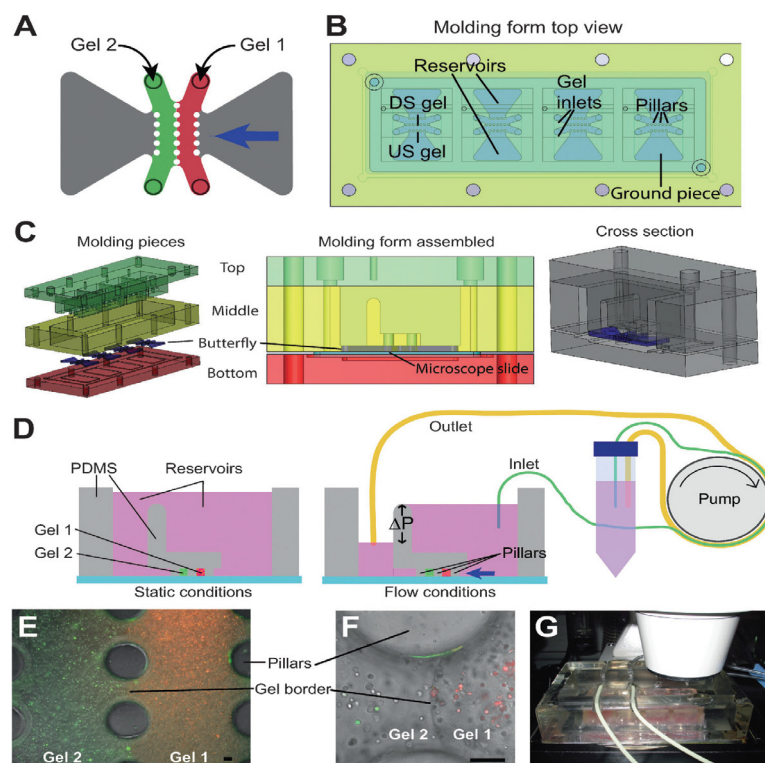




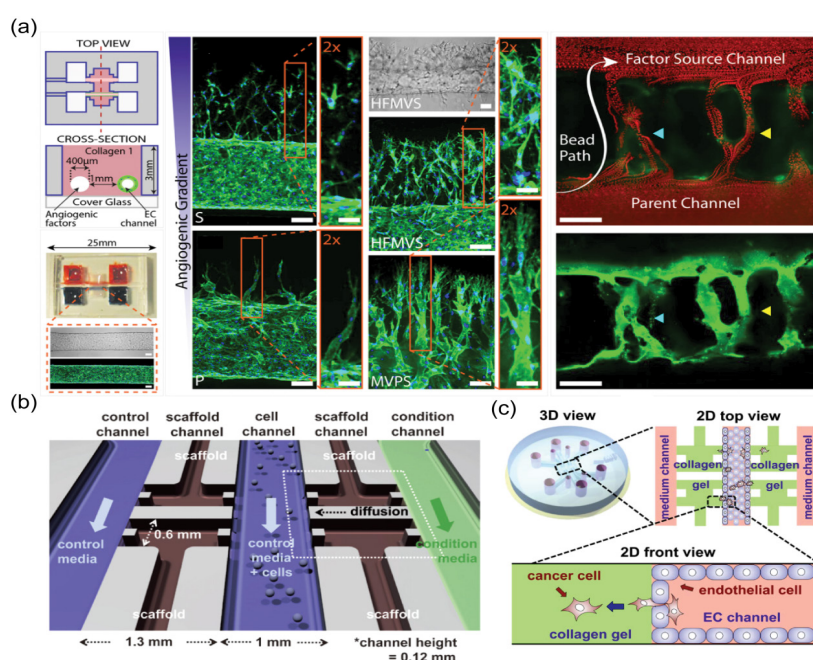
**Figure 2:** Common designs and features of the chips for cell-cell interactions. (a) Typical progress of seeding and culturing cells in 3D structure. (b) Pneumatic micro valve operations for cell co-culture. (c) Micro channel design for real time observation of cell-cell interaction. (d) Membrane insertion between two chambers for separating and culturing cells. Adapted from [68].



**Figure 3:** Laminar-flow control by microfluidics. (a) A device used for partitioning a microfluidic channel using gels to enable tunable 3D cell culture. Separation is achieved with laminar flow using a syringe pump [41]. (b) Patterning of cells using laminar flow [77].



**Figure 4:** Injection molding allows easy and robust set up of 4 parallel experiments on a conventional scope. (A) A single unit containing two gel compartments (upstream (US) and downstream (DS)) and medium reservoirs with different pressure heads that drive flow. (B) Top view of device shows four units on one slide, allowing parallel experimentation with a motorized microscope stage. (C) Flow chamber modules consisting of top, middle and bottom part defining the reservoirs and the butterfly-shaped ground piece creating gel channels in different perspectives as labelled. (D) Schematic of flow setup. For static conditions (left), both reservoirs are filled to the top; for flow conditions, a pressure head of 7 mm H<sub>2</sub>O is maintained throughout the experiment with a peristaltic pump setup as shown. (E) Fluorescence microscopy image overlaid with phase of the interface between two fluorescently-labeled cell-loaded gels confined by PDMS pillars. (F) Close-up of E showing the gel border. (G) Chamber setup with tubing casket on an inverted microscope. Scale bars: 100  $\mu$ m. Details can be found in [79].



**Figure 5:** Microfluidic 3D cancer models. (a) Three-dimensional formation of endothelial sprouts and neovessels in a microfluidic device [80]. (b) Schematic for microfluidic cell migration assay enabling direct comparison of cell migration behavior between the condition and control sides [81]. (c) Microfluidic system of tumor cell extravasation [82].

## Microfluidic applications in cell biology

Bio-microfluidics has been successfully applied in cellular biology for study various cellular behaviors and phenotypes including cell growth, differentiation, signal transduction, protein secretion and transport, gene expression, cell and ECM behaviors, and cytoskeletal dynamics.

**Microfluidic 3D cancer models:** a) Angiogenesis and metastasis: With the development of biomaterials, several chip-based models have provided information and data about cancer-related processes, such as angiogenesis, migration and extravasation (Figure 5). Angiogenesis comprises of endothelial sprouting and intussusceptive micro vascular growth, which is remodeling of existing vascular networks by forming a new vessel from an existing one. This process can be driven by growth factors, like vascular endothelial growth factor (VEGF) gradients, hypoxic conditions and the presence of tumors or trans endothelial flow. Angiogenesis is crucial to include in cancer models, because it is a prerequisite for tumor growth, invasion, progression, and metastasis. One strategy to build vascular models is to separate two parallel microfluidic channels by a hydrogel filled channel in the middle [80]. The other way is the vascular structures fully embedded within 3D ECM [83]. As an example of the second approach, endothelial colony-forming-derived endothelial cells and normal human lung fibroblasts are mixed with fibrin matrix [84]. Angiogenesis-based approaches were successfully employed in microfluidic devices to exploit paracrine and juxtacrine signaling between ECs and MSCs [75] or between ECs and fibroblasts to generate vascular networks [86]. It had been reported that EC and MSC co-cultures were employed to vascularize cell spheroids for therapeutic neovascularization [87].

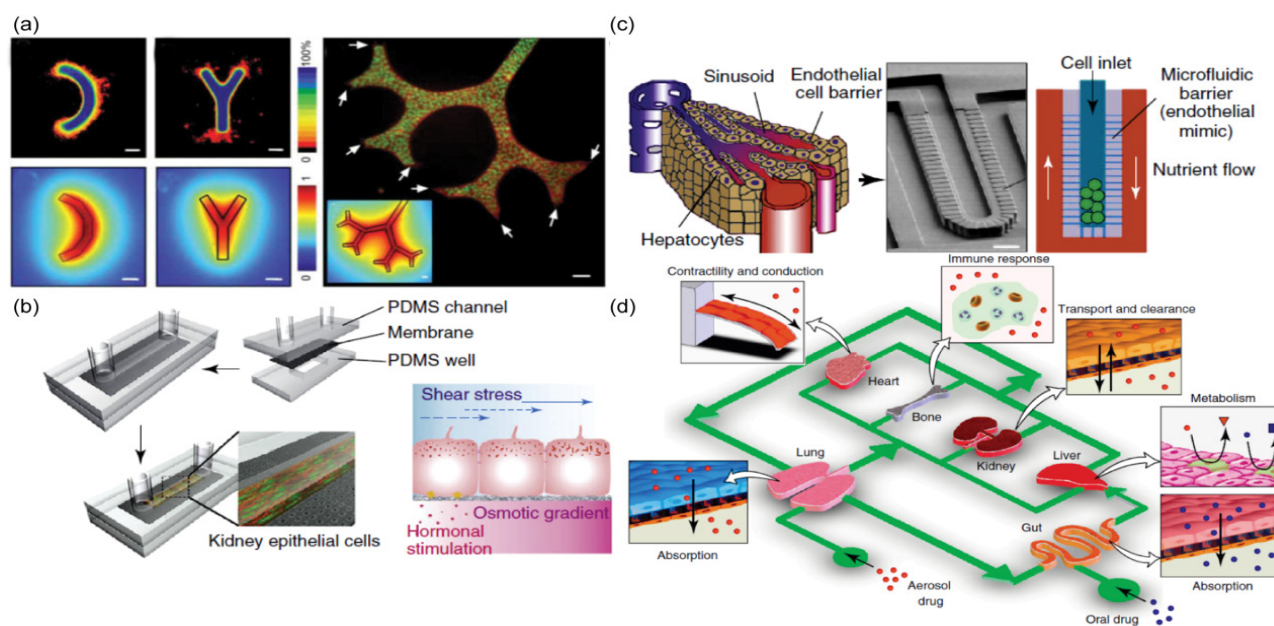
Metastasis is a complex process in cancer started with intravasation, invasion of cancer cells through the basal membrane into a blood or lymphatic vessel, followed by extravasation, entrance in other tissues and/or organs [16,88]. Chip-based models had been developed for intravasation and extravasation processes, in which the formation of an

endothelial monolayer on a 3D collagen type I hydrogel mimicking ECM enabled the precise quantification and control of critical microenvironmental factors.

b) Drug discovery on tissue or organ level: One of the successful practical applications of 3D cancer systems is in the drug discovery, especially for the screening of drug toxicity. Animal models are limited for difference in metabolism and cellular response to chemical signals from that in humans [89]. The 3D cancer systems provide a more humanized platform that may enhance the predictability of new drug in humans [90,91]. Multiple parameters in 3D microenvironment, which strongly affects the drug effect, require a more complex *in vitro* model to investigate at tissue or organ level.

Continued development and integration of microtechnology with 3D cancer biology support the generation of 3D *in vitro* cancer models at tissue level. The microminiaturization fabrication enables hollow shaped, or duct-like, structures, which are compatible with mammary duct and blood vessels. Nelson [92] created 3D mouse mammary ducts by micropatterning of collagen gel to investigate the effect of transformation growth factor-beta on mammary branching morphogenesis. They demonstrated the important role of tissue geometry during organ morphogenesis and its role in defining the local cellular microenvironment. However, this model lacks in introducing continuous flow. Other examples for duct-like structures that use microchannel geometry, gel patterning, fluid dynamics, or microfiber generation overcome this problem [83]. Bischel constructed a circular shaped lumen system after lining the lumen with endothelial cells. By a viscous fingering method, a less viscous solution tunnels through the center of a more viscous solution can be created [93,94]. organs-on-chips (also known as organ-on-a-chip) is microfluidic device for culturing living cells in continuously perfused, micrometer sized chambers in order to model physiological functions of tissues and organs.

The word chip in organ-on-chip stems from the original fabrication



**Figure 6:** Microengineered tissues or organs on chips. (a) Branching position is determined by tubule geometry and is consistent with the concentration profile of secreted diffusible inhibitor(s) [92]. (b) A microfluidic kidney epithelium model [95]. (c) Liver-on-a-chip [96]. (d) The human-on-a-chip concept [4].



method, a modified form of photolithographic etching used to manufacture computer microchips, which allows control of surface feature shapes and sizes on the same scale (nm to  $\mu\text{m}$ ) that living cells sense and respond to in their natural tissue milieu. The simplest system is a single, perfused microfluidic chamber containing one kind of cultured cells that exhibits functions of one tissue type. In more complex designs, two or more micro channels are connected by porous membranes, lined on opposite sides by different cell types, to recreate interfaces between different tissues. Development of these micro engineering approaches has opened entirely new possibilities to create *in vitro* models that reconstitute more complex 3D organ level structures and to integrate crucial dynamic mechanical cues as well as chemical signals. There have been different kinds of tissue or organ chip models for specific research (Figure 6), such as liver-on-a-chip, [96] kidney-on-a-chip, [97,98] gut-on-a-chip, [99,100] lung-on-a-chip, [101,102] heart-on-a-chip [103] and vessel-on-a-chip [104].

The integration of functional organ mimetics, such as gut-, liver-, lung- and skin-on-chip within a “human-on-a-chip” (Figure 6d), could provides improved methods to explore different routes of drug delivery (oral, aerosol and transdermal), as well as their effects on the efficacy or toxicity of different drug formulations [4].

Recently, a new platform has been developed called the tumor-microenvironment-on-a-chip (T-MOC) to mimic the complex pathophysiological transport within the tumor and surrounding microenvironment. The T-MOC system is able to precisely modulate environmental parameters such as interstitial fluid pressure and tissue microstructure to analyze the significant effects such as each parameter dictates on nanoparticle and drug transport [105,106]. Similar to T-MOC, there is organ-tumor-on-a-chip. Organ-tumor-on-a-chip refers to modeling of tumors on microfluidic chips, and the resulting organ-on-a-chip type human cancer models can be used for research on cancer growth and metastasis, drug target discovery, testing drug compounds, and for associated companion diagnostics. Investigations have been made recently on tumor-on-a-chip for the tumor of lung, bone marrow, brain, breast, urinary system (kidney, bladder and prostate), intestine and liver [107].

Microfluidic organs-on-chips research must also contend with specific technical challenges. Fabrication requires specialized micro-engineering capabilities. Bubbles in microfluidic channels may injure cells and hamper fabrication and control of chips, and it can be difficult to completely remove them. Although continuous perfusion generally supports high levels of long-term cell survival, the use of simplified ECM gels or thin ECM coatings can be a problem owing to matrix degradation or contraction over time. Additional challenges include achieving robust and consistent cell seeding in microfluidic channels, preventing microbial contamination, and controlling the cell-cell and cell-ECM interactions necessary to generate precise tissue structure-function relationships. Even with these limitations, microfluidic culture devices have much to offer. In organs-on-chips, cell types of one tissue can be positioned precisely and consistently relative to those of another. Another advantage of organs-on-chips is the ability to control fluid flow, which enhances the differentiation, function and long-term survival of many cell types. For example, human lung cells have been cultured on chip in a functional state for at least one month in culture [108]. The true power of microsystems engineering lies in the ability to design synthetic culture systems in which many different control parameters (e.g., types and positions of cells; precise 3D orientation of tissue-tissue interfaces; transcellular chemical, molecular and oxygen gradients; flow levels and patterns; mechanical forcing regimens) can be changed independently, while simultaneously carry out high resolution

and real-time imaging of molecular-scale events within a 3D tissue or organ context.

Although bioengineered 3D microsystems and organ-on-a-chip technologies are relatively new and still require further validation and characterization, their potential to predict clinical responses in humans could have profound effects on drug discovery and environmental toxicology testing. The scale-up of these complex technologies, together with systems integration of the engineering (e.g. fluidics handling, pumps) into easy to use, scalable, reproducible and user-friendly systems will be the key to their future success. It will be important to ensure that appropriate biomarkers and assays are developed for use with these microsystems, and to validate the extrapolation of *in vitro* results to the human situation.

Microfluidic devices for cell-based assays have provided new types of microenvironments and new methods for controlling and observing the cellular responses. As more microfluidic devices for cell biology are developed and implemented to address the current roadblocks such as ease of use, biological validity, and limitations in readouts, the unique strengths of these devices will become more accessible to the general biology community as common laboratory tools.

### Stem cell biology

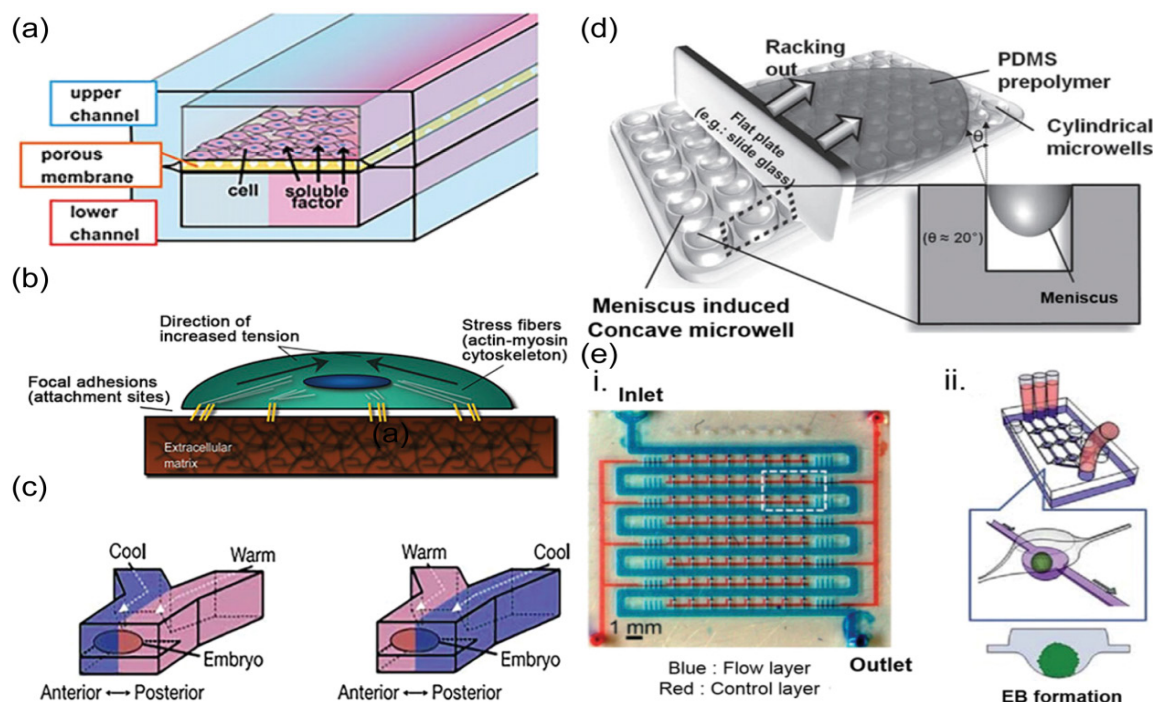
Stem cells *in vivo* are established in niche, which is the microenvironment stem cells lived in and preserves stem cells from physiological stimuli. This microenvironment regulates how stem cells participate in tissue generation, maintenance, and repair. Micro-technology-based platforms enable imitation of complex physiological context, which fulfill the requirements in the area of stem cell studies.

**Regulation of human stem cell differentiation:** As the interplay between stem cells and their microenvironments strongly influences stem cell differentiation, microfluidic control of soluble and insoluble factors enables precisely anticipate of the cell fate. The ways to control these factors are discussed in section 2 Cell and environment control, which are similar in regulation of stem cells. In this part, we will present how these factors affect differentiation of stem cells.

Kawada presented a membrane-based microfluidic device designed to form spatiotemporally nonuniform culture environments for stem cells (Figure 7a). The soluble factors diffused through the membrane depending on flow rate. In their work, the soluble factors include RA and leukemia inhibitory factor were introduced to determine whether miPSCs expressed Nanog, a transcription factor required for self-renewal of ESCs. The results showed that a low concentration of RA for 72h induction was sufficient to suppress Nanog.

The other way to control soluble factors is to generate concentration gradient with a slow flow rate by using a simple osmotic pump. Stem cells in the chemical gradient experienced different fates [113]. Using soluble factors, Kim proposed a microplatform for on-chip differentiation of embryoid bodies [114]. They changed the duration and rate of the flow to adjust the average size of embryoid bodies, and then applied RA to induce differentiation of EBs into a neuronal lineage. The results showed that RA-treated cells appeared as mature neuronal cells with long neurites in a relatively intense response to the neuron-specific antibody TuJ1. This microsystem had been proven to be useful on-chip method to induce and monitor differentiation of EBs.

Mechanical forces like stiffness and shear stress provide a crucial set of signals to alter the structures of cells and regulate their functions, resulting in a change of differentiation. Stiffness of substrates determines the stiffness of cytoskeletons (Figure 7b). Gilbert demonstrated the



**Figure 7:** Microfluidic applications in stem cell biology. (a) Soluble factors are applied to stem cells as fate determinants through porous membrane [109]. (b) Physical factors applied as fate determinants include mechanical stimuli such as stiffness [100]. (c) Other factors applied as fate determinants include temperature [38]. (d) Static methods used for EB formation. (e): Dynamic methods used for embryoid body (EB) formation. (ei): Optical image of microfluidic EB trap arrays [111]. (eii): Schematic of EB formation within microwells in a multilayer microfluidic platform [112].

effect of stiffness on muscle stem cells (MuSCs) in a bioengineered microplatform, and they found that soft substrates with moduli of 12 kPa were observed to be better for self-renewal of MuSCs and muscle regeneration [115]. Modifying substrate stiffness is the way actually changes the interaction between cells and substrates, would also orient the differentiation of NSCs. By use of variable moduli interpenetrating polymer networks to modulate the stiffness of substrate, researchers found that moduli of approximately 10 Pa blocked the differentiation, while moduli of about 500 Pa induced expression of the neuronal marker  $\beta$ -tubulin III and differentiation into glial cells with moduli of approximately 1,000–10,000 Pa [116].

Shear stress also plays role in regulating the differentiation. Lee controlled shear stress by microfluidics and found that ESCs differentiated into endothelial cells under the uniform cell docking and shear stress conditions [117]. Kim investigated the osteogenic differentiation of MSCs according to the applied shear stress through transcriptional coactivator with PDZ-binding motif (TAZ) activation using a microplatform with an osmosis-driven pump [118]. The interstitial level of shear stress induced osteogenic differentiation of MSCs, which is inherently caused by nuclear localization of TAZ.

Temperature has been used on chip to control the behaviors of stem cells by Lucchetta's group (Figure 7c). They placed an embryo in a micro platform and introduced a flow with different temperature steps to the anterior and posterior halves of the embryo [38,119]. Temperature was shown to significantly affect the early development of the embryo. Other factors include dissolved oxygen, compressive pressure and electricity also proved to have influence on stem cell differentiation [120–122].

Before the differentiation of ESCs, the formation of embryoid bodies (EBs), three-dimensional aggregates of pluripotent stem cells, are regulated by homophilic interactions of the  $\text{Ca}^{2+}$ -dependent cell

adhesion molecule E-cadherin [123–125]. The size of the EB is a critical factor influencing the differentiation of ESCs: Large EBs tend to become part of the endoderm and mesoderm, while small EBs tend to differentiate into ectoderm tissue. There are two main types of methods to control EBs. One is static, using well-defined micro wells to form EBs and to control their average size [126,127] (Figure 7d). The other way is dynamic (Figure 7e), which use microfluidics to control EB size easily and enables researches under various changes of the microenvironment [114].

**Stem cell niche engineering:** A 3D microfluidic culture model is required to recapitulate the native niche. There are various of microtechnologies (e.g. stop-flow lithography [128], robotic formation of micro gels [26] and droplet microfluidics). And droplet microfluidics is capable to compartmentalize cells into mono-dispersed and physic chemically defined 3D matrices, therefore, is advantageous in constructing artificial niches. Both synthetic and natural hydrogels have been utilized in creating in vitro 3D platforms by mixing different cells into these hydrogels in varied mechanical and biochemical gel properties. For example, Huck and colleagues tuned the content of collagen/gelatin and the degree of cross-linking to obtain microbeads with varying elasticity and biodegradability. These beads were then used to study fibro-blast invasion into matrices of different stiffness [129]. Similar studies have been processed with synthetic hydrogels. Rossow's group used hyper branched polyglycerol and PEG and controlled micro gel elasticity by the molecular weight of the PEG cross-linker and the precursor concentration. They realized encapsulation of lymphoblasts and fibroblasts within microcapsules obtained through the co-polymerization of these materials [130]. Garcia's group employed PEG-maleimide-based microgels for successful long-term cell culture, showing high viability of human MSCs, as well as insulin secretion of human pancreatic islets [131]. 3D co-culture for niche construction can



be realized with droplet-based technology. By varying the flow rate ratio between the two cell streams, the ration between the concentrations of two cell types can be altered within the micro gel [132]. Another way to build co-culture is the layer-by-layer. Sakai and colleagues reported a first microfluidical encapsulation of rat adipose-derived cells in gelatin microbeads, and a co-encapsulated in another gelatin microbead, which supported the adhesion of L929 cells [133].

All these examples highlight the advantages of droplet microfluidics to generate cell-laden microcapsules as cell-instructive microenvironments.

## Conclusion

The progress in microfluidic culture, especially the new microfluidic 3D model, elucidates questions in cell biology in a more efficient way. Because multiple key biophysical and biochemical parameters attribute to the complexity of in-vivo microenvironment and are controllable by 3D microfluidic devices.

With more vivo-like features, researches based on 3D microfluidic culture model other than 2D model may be a trend in cell biology studies to provide a greater understanding of biological mechanisms and better guide the design of more physiologically compatible systems. The recent development of 3D microfluidic culture in tissue engineering has resulted in the evolution of 3D in vitro models for cell biology studies [134]; meanwhile, it calls for the development of tissue engineering and biomaterials to maximize the utility and functionality of the models. For example, smartly designed biomaterials can be degraded by growth factors at desired rates that relevant to physiological condition or be cleaved only by specific proteases like *in vivo* ECM [135-137]. Several pharmaceutical companies are moving toward adapting 3D in vitro cancer models as anti-cancer drug testing tools. However, it does not mean that complex organ-level in vitro culture systems will be a dominant strategy. In studies that focus on cell interaction, relative simple models are needed to avoid multiple interferences. These models are widely used in the early stages to identify therapeutical targets or screen drug candidates. For later steps like exploration of drug toxicity, analyzing at tissue/organ levels may be necessary, because multiple parameters would affect toxicity on cells. Widespread use of the microfluidic culture systems in practice would be challenged by the limitation of reliable detection methods for high-throughput and high-content analysis. It is important to develop methods for imaging, detecting, and quantifying signals. Despite this challenge, the continued development and integration of micro fluidic culture, tissue engineering and biomaterials will bring significant contributions toward a deeper understanding of mechanism in cell biology.

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