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REVIEW ARTICLE

Microfluidic Platforms for the Study of Cancer Metastasis

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Abstract
Metastases many a time leave cancer patients untreatable and are one of the leading causes of death worldwide. Microfluidic platforms are arguably the most suitable for the study of cancer metastasis given its ability to mimic *in vivo* microenvironment of cancer tumor by manipulating its mechanical properties. This review discusses some applications of microfluidic platforms and their advantages for cancer biology and pathology. Studies of cancer metastasis conducted on its compositional steps enable us to elucidate elementary mechanisms through disease modeling. From that, communication and interaction of cancer cells, cellular metabolism related issues, and ultimately cancer drug discovery and delivery are manipulated on microfluidic platforms.

Keywords Cancer metastasis, Microfluidics, Microenvironment

Abbreviations

2D	two-dimensional
3D	three-dimensional
ECM	Extracellular matrix
EGF	Epidermal growth factor
HEC	Human endothelial cell
hEGC	Human embryonic germ cell
VEGF	Vascular endothelial growth factor

INTRODUCTION

Cancer is known as a leading cause of death globally. Each year, more than 12.6 million people are diagnosed with cancer and it accounts for at least 7.6 million deaths [1]. Among this large number of death toll, most of them are due to metastasis, namely, the spread of cancer cells from its primary site to other organs. Metastasis itself is a complicated and dynamic process involving angiogenesis, tumor cell proliferation and dissociation, surrounding stromal invasion, migration, intravasation, circulation, extravasation, and micrometastases formation. In the process, a number of interactions among tumor cells and between tumor cells and the host tissue take place [1, 2]. During tumor progression, normal cells transform to hyperplastic through dysplasia, carcinoma *in situ*, invasive and finally metastatic. They adapt and undergo genetic changes as well as proteomic changes in the process [3]. From that, discerning the foundation mechanism of cancer cells metastasis may provide insights for successful treatment of the disease [3]. Fig. 1 shows the foundation mechanism of the process of metastasis.

To understand this heterogenic and dynamically evolving molecular ecosystem, many assays have been developed over the decades [4-6]. Among conventional models, the most commonly adopted one is based upon Boyden chamber-Transwell assay, which studies the transmigration of cancer cells across endothelial cells [7]. A modified Boyden chamber-Transwell assay was utilized for the study

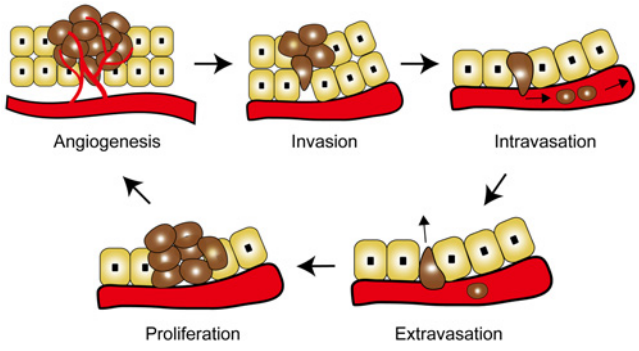

Fig. 1. Metastasis. Schematic diagram of the foundation mechanism of cancer cell angiogenesis, whereas cancer cells invade, following by intravasation into either blood vessels or lymph ducts, extravasation from either blood vessels or lymph ducts to other tissue, and proliferation of cancer cells on the newly located tissue.

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of tumor cell invasiveness by seeding the cells of interest on one side of a chamber while introducing a chemoattractant on the other side with a membrane which favors transmigration in between the two sides [8-11]. Although this device is able to mimic tumor cell invasiveness, it is unable to provide for the analysis of underlying influence by biophysical factors such as fluid convection and shear stress that could also be contributing to transmigration, let alone reconstituting complex 3D structure.

3D Assays utilizing hydrogel to examine dynamic mechanical environment in the role of regulating cell function were developed [12-19]. Containing a large amount of water, hydrogel is similar to natural ECM and can be modified to imitate chemical and mechanical properties of natural extracellular matrix (ECM). Other than that, we can also mimic the interaction of ECM and cells by various manipulations like dissolving a certain part or reorganize the structure [20].

Other than the manipulation of microenvironmet, microfluidics provides many advantages and is suitable for the study of cancer metastasis as its scale is comparable to the intrinsic dimensions of cells and capillaries. Furthermore, the flow in microchannels is almost always laminar and mass transport is dominated by local diffusion whereby microchannels can be used to create concentration gradients having complex profiles [21, 22]. Other than that, microfluidic platforms provide both two-dimensional (2D) and three-dimensional (3D) biochemical gradients and allow for precise control of biochemical gradients along with high quality analysis and time-lapse imaging. Taking these characteristics into accounts, it is beneficial for the study of cancer using microfluidic platforms. Microfluidic platforms act as useful and versatile tools for the study of cells for various biological Applications [23]. For one, the recreation of cancer models on chips is more realistic and the compartmentalization of cancer cell microenvironment enables the study of size and spatiotemporal dependent effects of metastatic cancer cells. Tuning the aspects of interest, we could investigate their correlation and a variety of investigation could be conducted either it is chemical manipulation, 3D analysis or real time tracking.

By breaking down the whole process of metastasis into its compositional steps and by integrating two or more of those steps, microfluidic platforms enable us to manipulate the *in vitro* microenvironment in order to demystify metastasis as a whole and each of its compositional elementary mechanisms.

CANCER METASTASIS MODEL

Cancer cells are highly sensitive to their microenvironmental stimuli. Their migration mechanisms adapt to the surrounding environment more effectively than non-cancer cells do [24]. In that sense, metastasis can be modeled as a mechanical process where it has to break from the primary cancer site and migrates. The spreading of cancer cells commonly travel through either bloodstream or lymph system. Chemical communication, the interference between and among cells through chemical signaling happening in cancer environment, plays an important role in metastasis. Similarly, the physical stimuli in cancer environment such as interstitial flow cannot be ignored [25]. Cellular microenvironment refers to continuous and reciprocal cell-cell interaction and cell-3D matrix interaction in the tumor microenvironment and is responsible to promote cancer progression and invasion [26]. Other than the three elements mentioned above, cellular metabolism is also an issue worth investigating, as cancer cells undergo cellular metabolism as well and show different pattern than the one of normal cells [27, 28]. Ultimately, as clinical applications of disease models utilizing microfluidic platforms, anti cancer drug discovery and delivery have been studied and are anticipated to play a main role in the development of personalized cancer treatment.

CANCER ENVIRONMENTAL STUDY

Chemical communication

Cancer cells interfere with other tumor cells, stromal cells and ECMs in every sub step of metastasis. They adapt themselves to the surrounding microenvironment during the multistep process by the means of paracrine and autocrine communication [29].

A study was conducted on the effect of autocrine epidermal growth factor (EGF) receptor on endothelial cell migration and vascular morphogenesis induced by vascular endothelial growth factor (VEGF) under interstitial flow. It was proved that low interstitial flow promotes growth, high survival and development of a confluent endothelial monolayer. Autocrine EGF integrates VEGF signaling which promotes capillary morphogenesis under interstitial flow [30]. Recently, there was also a study in progress regarding the effect of VEGF and the integrated gradient of ANG-1 to the morphology and characteristics of angiogenic response [19].

Another group seeded human epithelial ovarian cancer cell line (SKOV3) and human embryonic germ cell (hEGC) into separated reservoirs and subsequently developed them respectively in the two cell culture areas with communication through barrier channels. The study resulted in the inhibition of SKOV3 cells proliferation by hEG cells and that positive signals gradually decreased along the perfused medium flow [31]. Other than that, an analysis of the paracrine loop between lung cancer cells and fibroblasts was presented [32]. Using a microfluidic cell culture chip equipped with pneumatic microvalves, it is verified that cytokines from cancer cells

effectively stimulate fibroblasts into myofibroblasts and the cytokines from myofibroblasts rather than fibroblasts increase the migration speeds of cancer cells [32].

Microfluidic technology is being well utilized in the study of the mechanism of various forms of chemical communication, cell response to chemical gradients and the evaluation of drug therapy under different stimuli. Through the basic microfluidic technology, namely mixing, gradient, dilution, separation and many more, researches to answer more complex questions are lively under progress.

Physical stimuli

The biggest advantage of microfluidic platforms is their ability to control pressure and flow, induce gradients, and introduce multiple cell types while getting high-resolution imaging and quantification out of it [23]. Carefully manipulating those aspects of interest, interstitial flow across ECM, interstitial convection and diffusion in normal and neoplastic tissues regarding the effect of fluidic environment on cancer metastasis were carried out.

Interstitial flow is the convective transport of fluid through tissue ECM and it has been shown to play an important role in the morphology, function and migration of cancer cells. By applying a stable pressure gradient and fluid flow across a 3D collagen scaffold, the effects were studied [33]. It is found that high pressure from clustered tumor tissue induces interstitial flow and the strong interstitial flow nearer to tumor margin is responsible for tumor cell clustering and growth while the weak interstitial flow further from tumor margin is responsible for the spread and metastasis of cancer cells. This result is acquired thanks to the key feature of microfluidic platforms whereas interstitial flow between ECM and cells cultured 3 dimensionally is precisely controllable and quantifiable. The migration dynamics of breast cancer cells was investigated in a different type of tunable 3D interstitial flow chamber [34]. From that, it is found that interstitial flow increases the percentage, speed and persistence of migration in 5-20% subpopulation of the cells.

Other than visualizing mechanism of cancer cell pathology, one can obtain numerical results regarding relative metastatic potential of cells of interest. One of them is to quantify morphogenesis of human umbilical vein endothelial cells (hUVECs) under a flat, less steep or steep gradient [35]. As regards, microfluidic technology is advantageous in regulating physical stimuli and there are recently many studies conducted on integrating various physical stimuli, such as surface structure, electric field, magnetic field, strain, stress and stretch.

Cellular microenvironment

Interaction between cancer cells, cancer cells and stromal cells; and also the interaction between cells and ECMs contributes to each sub steps of metastasis from tumor cell dissociation to adhesion and angiogenesis.

For cell-cell interaction, a study on the response of endothelial cell placed in co-culture with physiologically relevant cell types including cancer cells or smooth muscle cells was conducted and resulted in the observations of cancer cells either attracting endothelial cells and inducing capillary formation or having minimal effect when compared to the suppressed endothelial activity by smooth muscle cells [36]. Nevertheless, the study is still incipient due to technical challenges like in the proper selection of cell culture condition and separation of cells. An innovative microfluidic technology was introduced in the mimic of microenvironment of gradients present in native tumors [37]. Tumor cells were formed in chambers, which were exposed to medium perfusion on one side to create linear nutrient gradients. Time-lapse microscopy was performed for the measurement of growth rate and the study of drug diffusion into tumor mass in a microenvironment similar to *in vivo* tumors.

The mechanism of migration, metastasis, morphogenesis, plasticity and EMT, which are strongly influenced by tumor microenvironments, can be further studied by the use of microfluidic platform. At any rate, it is essential to fully understand how cancer cells turn either less sensitive or resistant to cancer therapy while adapting to their surrounding environment in the process of metastasis. A 3D construction of more complex and heterogeneous tumor microenvironment is therefore needed for the observation and analysis of cell-cell interaction and cell-ECM interaction. Making precise co-culture of various cells and control of mechanical and chemical stimuli possible, microfluidic technology is crucial in the study of metastasis.

CANCER METABOLISM STUDY

Undergone cellular mutation, cancer cells have their own sets of mechanism in cell division. Similarly, their cellular metabolism pattern is of much difference than the pattern of normal cell metabolism. The hallmark of cancer cell metabolism is hypoxia, which is a metabolism transformation when tissue growth outpaces the speed of blood vessels growth. Subsequently, this alters energy production mechanisms and nutrient uptake by cancer cells [27, 28].

A study of the effect of hypoxia on angiogenesis was studies utilizing microfluidic platforms. Mimicking hypoxia on chip, the kinetics of cell-cell interactions was found to have turned into a one-way traffic. Only migration of endothelial cells toward tumor cells was observed but not any migration of tumor cells toward endothelial cells. This suggests that hypoxia suppresses the migration ability of

tumor cells and prompts release of angiogenic growth factor to attract endothelial cells for angiogenesis [38, 39]. Nevertheless there are still many argues regarding this. Another group studied the interrelation of hypoxia and VEGF response in tumor biopsies and reached the conclusion that hypoxia induced a greater VEGF response in tumor biopsies than that of non-cancer tissues [40].

Though known to be a very important issue in cancer metastasis, the correlation between hypoxia and cancer has not yet been vastly tested on microfluidic platforms but it is anticipated to be a hot subject of study in the field of cancer study soon. Taking cancer environment and metabolism into account, we could mimic a more realistic disease model with the use of microfluidic platforms and ultimately discovery cancer drug and study its delivery as well.

CANCER DRUG DISCOVERY AND DELIVERY

The current clinical approach to cancer treatment is often regarded as "trial and error" or "one size fits all". Cancer is still treated with a generalized methodology regardless of the

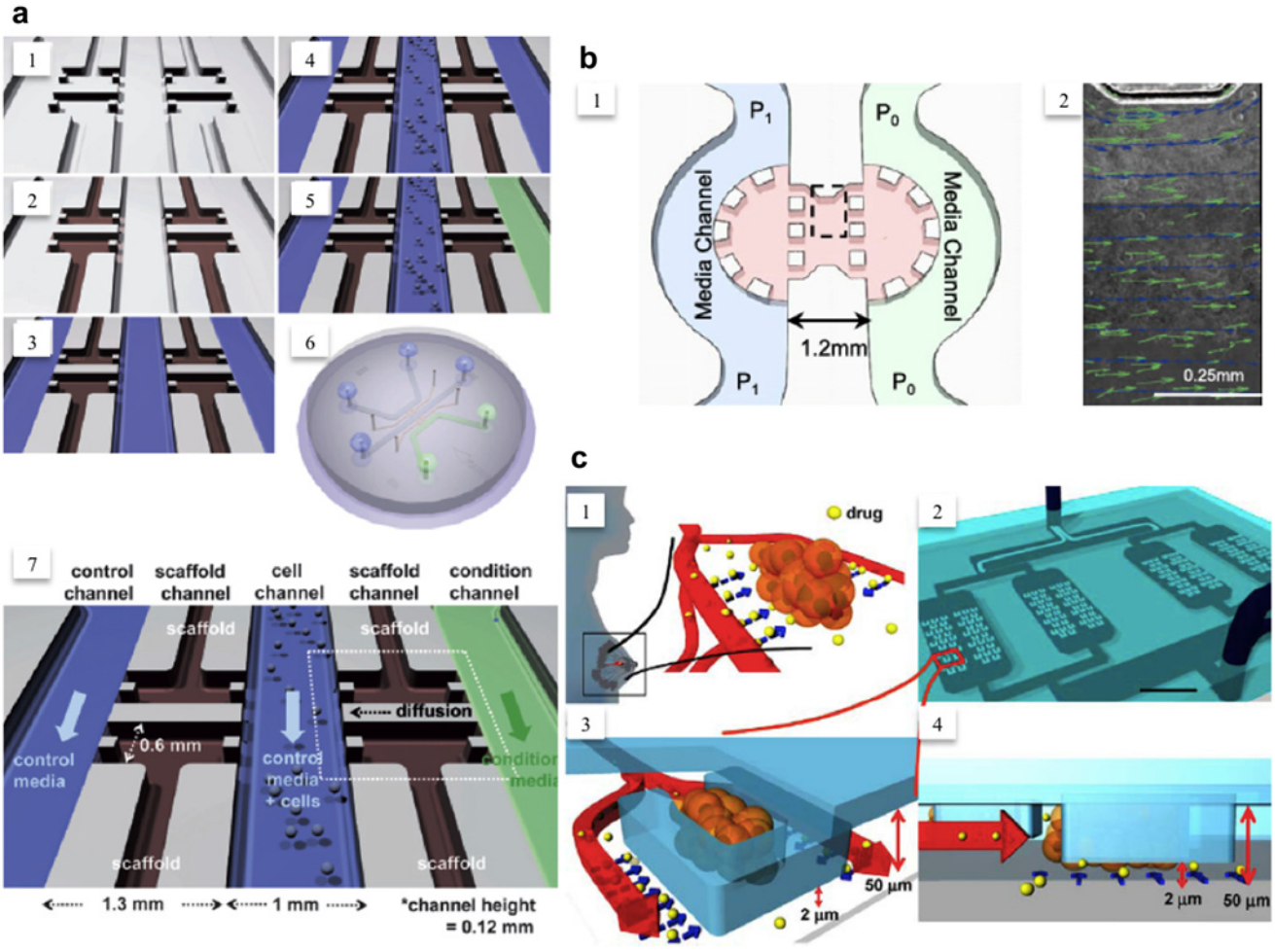

Fig. 2. (a) Schematic of experimental settings for co-culture conditioned microfluidic assay development. (panels 1-6) PDMS device was made by soft lithography and surface treatment, whereas gel scaffold was filled within (brown) scaffold channel between (blue) media channels. (panel 7) With cell (spheres) seeded in the central cell channel and chemical factor in the condition (green) channel, microfluidic cell migration assay enables direct comparison of cell migration behaviors between the control and conditioned channels. Droplets were placed on all reservoir outlets to avoid evaporation (panel8) [36]. (b) Illustration of microfluidic cell culture system with 2 two channels (panel 1) to investigate the effects of interstitial flow on tumor cell migration. A consistent flow field was generated by applying a constant pressure across the gel. (panel 2) Velocity vectors were tracked with fluorescent microspheres (green) superimposed on streamline vectors (blue) for a computation model [33]. (c) Schematic diagram of microfluidic spheroid array where *in vivo* tumor spheroid is characterized by tumor cells with drug molecules coming from nearby blood vessels during drug dosing (panel 1). Diagrams of the platform (panel 2) with 7,500 U-shape traps per square centimeter, the enlarged view of one of the U-shape traps (panel 3) and the side view of the trap to illustrate perfusion channel (panel 4) were shown. [44].

high specificity of each patient. This is inefficient and frequently results in inappropriate or ineffective therapy. In contrast, personalized treatment has the potential to overcome the problems stated above [41]. From this, microfluidic platforms are very useful in mimicking personalized microenvironment that would be different for each patient.

It is now increasingly recognized that cell motility and invasion might provide new targets for cancer therapy and that appropriate inhibitors may restrain both metastasis and neoangiogenesis. This calls for the need of platforms on which cell movement and cancer metastasis ability can be measured rapidly and quantitatively [42].

With the ability to dynamically control the microenvironment, temporally varying drug profiles to mimic physiologically measured profiles were made possible. In addition, the link between hypoxia on cell response to drug treatment was made possible by inducing oxygen concentration gradients [43]. Other than stimulating drug gradients and oxygen concentration gradients on chip, another approach to test for anticancer drug was by forming cancer cell spheroids as a platform for anticancer drug assays. The formation of spheroid was based on hydrodynamic trapping of cancer cells in controlled geometries and the size and speed of spheroid formation were found to be manipulable through its flow rate [44].

Different from testing the drug effect *in vitro*, the concept of *in vivo* drug delivery involves a stable sensor platforms embedded into a patient's cancerous site in order to deliver the quantities of drugs that are clinically necessary over a comparatively long period [45].

Along with the advances in microfluidic platforms and the increasing vast applications of microfluidic platforms in the study of cancer, we could envision a total personalized cancer therapy starting with the duplication of cancer microenvironment of a patient onto a microfluidic platform, the testing of suitable cancer drug on it and finally the embedding of microfluidic based drug delivery platform into the particular patient.

CONCLUSIONS

Microfluidic platforms serve as a means with a wide range of customization in mimicking the intrinsic characteristics of *in vivo* cancer microenvironment. Utilizing the platforms, we could perform time-consuming processes in a single chip. From that, we benefit in the reduction of reagent waste, time loss, work labor and the use of animal study whether it is in the study of the basic mechanism of each sub steps of cancer metastasis, the study of the correlation of tow or more of cancer compositions or in its application for the use for cancer drug discovery and delivery.

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