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Microfluidic Device with Dual Mechanical Cues for Cell Migration Investigation

Chin-Hsiung Tsai and Po-Ling Kuo

Abstract—**Cell migration plays an important role in numerous physiological and pathological conditions, such as angiogenesis, wound healing and cancer metastasis. Understanding the fundamental mechanisms of cell migration is crucial to develop strategies for disease treatment and regenerative medicine. Several biomechanical cues have been well studied about their effects on guiding cell migration. However, the effects of dual or multiple cues on cell migration are barely addressed. In this work, we developed a microfluidic-based device to study the combinatory effects of osmotic and stiffness gradient on cell migration. Computer simulation and experimental validation showed that the device was capable of providing stable osmotic and stiffness gradient to cultured cells at the same time. Preliminary results suggest that our device has a valuable potential in studying cell migration in complex conditions which better recapitulate the complex environmental conditions *in vivo*.**

I. INTRODUCTION

Cell migration play a critical role in numerous physiological and pathological processes, such as tissue development, wound healing, angiogenesis, and tumor metastasis. Understanding how cells move in response to various environmental conditions should shed light in numerous medical issues such as regenerative medicine and cancer invasion. A growing body of evidence shows that mechanical cues, including osmolarity and substrate stiffness, are important to guide cell migration. For example, osmotic pressure has been showed to be an important signal to guide cell migration [1] and control cell motility [2]. Substrate stiffness is another important mechanical stimulation to guide cell migration and has been studied extensively [3, 4].

Although these studies have shown deep insights to explain the relation between one specific signal and cell migration, there are still few have built an environment providing dual or multiple gradient cues simultaneously [5]. Understanding the cell behaviors under a more in vivo-like situation will further extend our knowledge to cell migration and have more reliable information for medical application. However, this idea is mainly limited by the nature that one method can usually generate only one well-controlled signal.

In this study, we propose a new approach that integrates two different systems into a dual cues device which can generate substrate stiffness and osmotic gradients simultaneously. The two systems in this device are: (1) a gradient-compliant PA gel which is responsible for the first mechanical cue, the substrate stiffness gradient, and (2) a microfluidic system which can generate the second mechanical cue, the osmotic gradient. The gradient is built through the diffusion of sucrose from source to sink and can be maintained for long term cell culture studies. To develop our dual cues device, we have created two new steps to embed the PA gel in the microfluidic system and protect the gel from damages during the conventional poly(dimethylsiloxane) (PDMS) microfluidics fabrication process.

II. DEVICE DESIGN AND OPERATION PRINCIPLE

A. Device design

The main components of the device are shown in Fig. 1, a. The microfluidic channel includes the main channels and inlet channels (Fig. 1, b) for providing fresh cell culture medium, removing wastes and generating osmotic gradients. PA gel is used to provide stiffness gradient. The Glass_1 is used to hold PA gel and overcome the challenge that PA prepolymer solution is difficult to polymerize on PDMS. The square groove on PDMS substrate is for Glass_1 and PA gel to be embedded in. The main function of Glass_2 is holding PDMS substrate to ensure the surface of groove on PDMS substrate is smooth, it's more important for thin PDMS substrate (i.e. less than 600 μm). The inlet is connected with a syringe pump, and the outlet is a one side of the main channel (Fig. 1, c). The dimension of each component is shown in Fig. 1, d.

B. Operation principle

The operation principle of the dual mechanical cues device is illustrated in Fig. 2. The region where inlet channels connected to the main channel is the source side for high osmolarity cell culture media, which composed of 10% FBS and 1% antibiotics in Dulbecco's Modified Eagle's Medium with high concentration of sucrose, and the outlet of the main channel is connected to the bulk solution where is sucrose-free cell culture media (Fig. 1, a and Fig. 2, a). Because the flow velocity driven by the syringe pump is small ($\fallingdotseq 1\times10^{-9}$ m/sec) in the main channel, the distribution of sucrose would be similar with the result from diffusion, thus creating a concentration gradient in the direction from inlet to outlet. The cell migration experiment is performed in the cell culture chamber composed of the main channel and a compliant-gradient PA gel. The directions of the two simulations, stiffness and osmotic gradients, in the cell culture chambers are shown in Fig. 2, b, and they are perpendicular with each other. However, it is flexible to change the relative direction between them dependent on the requirement of different experiments. Fig. 2, c shows the concept about how the two signals simultaneously stimulate a cell in the cell culture chamber. The cell can not only sense the osmolarity

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difference between the front and the back, but also stiffness difference between the left side of the cell.

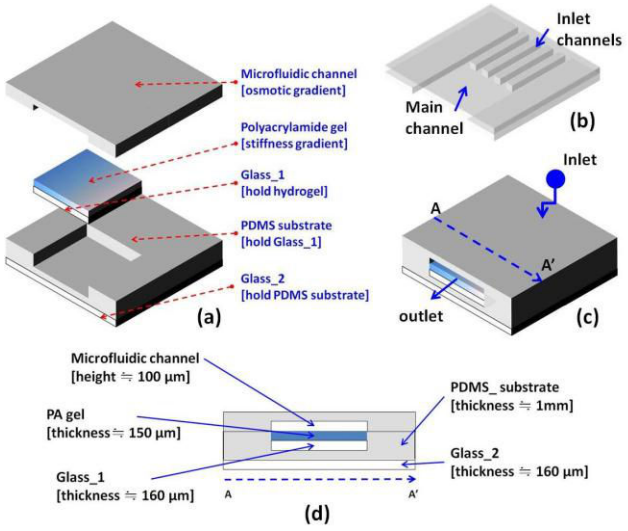

Figure 1. Schematic of the dual mechanical cues device (not to scale). (a) Main components and their functions (in square bracket) of the device. (b) Perspective view of the top layer, the inlet channels and the main channel. (c) Positions of inlet and outlet, the blue circle represents the syringe pump. (d) Cross section view of the A to A' line in (c).

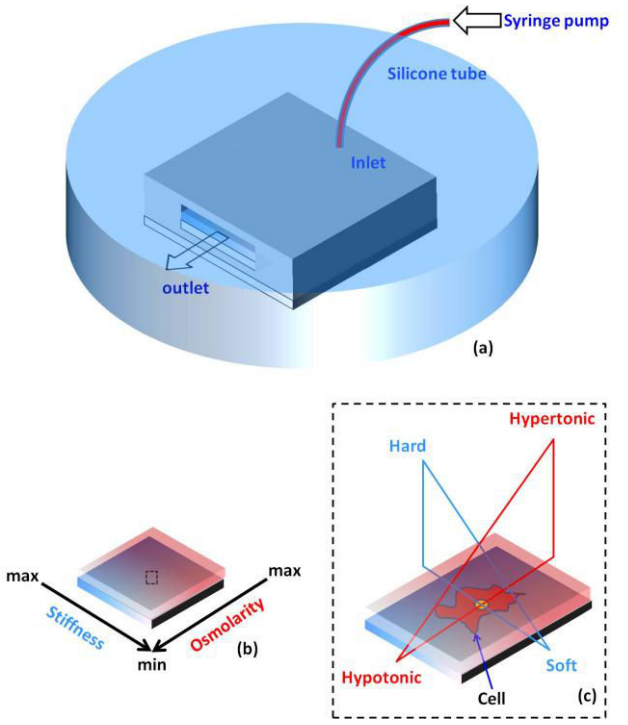

Figure 2. Schematic of device operation, dual gradients and signals sensed by the cell (not to scale). (a) The whole device, except the inlet tube, is immersed in the standard cell culture media. (b) The color changes indicate the intensity distribution from maximum to minimum of the two stimulations in the cell culture chamber. (c) Enlarged box in the middle of (b) shows the two different gradient signals sensed by the cell attached on the PA gel.

III. METHODS

A. *Integration of the PA gel and the microfluidic channels*

The integration process of the PA gel and the microfluidic system (Fig. 3) started from assembling Glass_1, PDMS substrate and Glass_2. We used oxygen plasma bonding method and it would render the PDMS surface to be hydrophilic. Therefore, we had to cover the non-bonding area with 3M tape to retain their hydrophobicity, which prevented chemical solutions spreading from the groove during the surface modification process of Glass_1. The polymerization method of gradient-compliant PA gel is slightly modified from Engler's protocol [6]. The final bonding process (Fig. 3, c) was completed as fast as possible to decrease the possibility to dehydrate the PA gel, and we also use a customized coverslip (5 mm x 5mm) to protect the PA gel from reactive ion species. The fastest bonding condition (18W, 890 mtorr, 33 sec) we had was achieved through performing the air plasma activation under higher process pressure. The device was filled with deionized water to prevent the PA gel from dehydration, some bubbles might appear during this process. After sterilizing the device with UV light, we immersed it in deionized water overnight to remove bubbles, then injected the 1-(3-(dimethylamino) propyl)-3-ethyl-carbodimide methiodide (ETC) solution through the inlet to perform the protein-PA gel conjugation process (Kandow, Georges et al. 2007). The functionalized device was ready to be used in cell culture experiments or kept in PBS at 4℃.

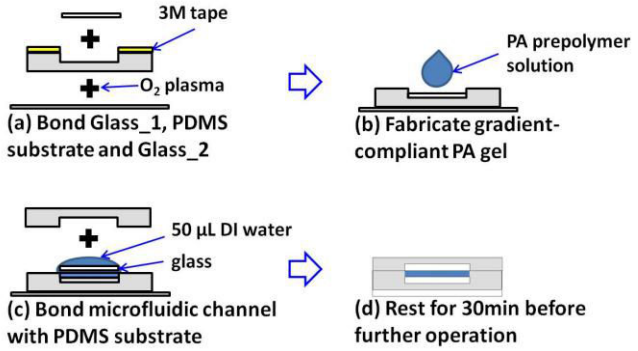

Figure 3. Fabrication process for integrating microfluidic system with PA gel.

B. *Simulation of osmotic gradients*

COMSOL Multiphysics 4.2a was used to simulate sucrose distributions in the main channel. The fluid was defined as incompressible flow and neglect inertial term. The diffusion coefficient of sucrose was defined as 5 x 10^{-10} m^2/s for free flow media. All the results were presented in the equilibrium state except the concentration-time dependent change. For the top view to the cell culture chamber, we coupled two physical models, laminar flow and transport of diluted species, to perform the sucrose concentration and flow profile in the free flow area. For a more specific simulation to our device, we used the other two physical models, Brinkman equations and species transport in porous media. This model construction could give us a more realistic prediction since the substrate in the cell culture media was PA gel, a porous medium.

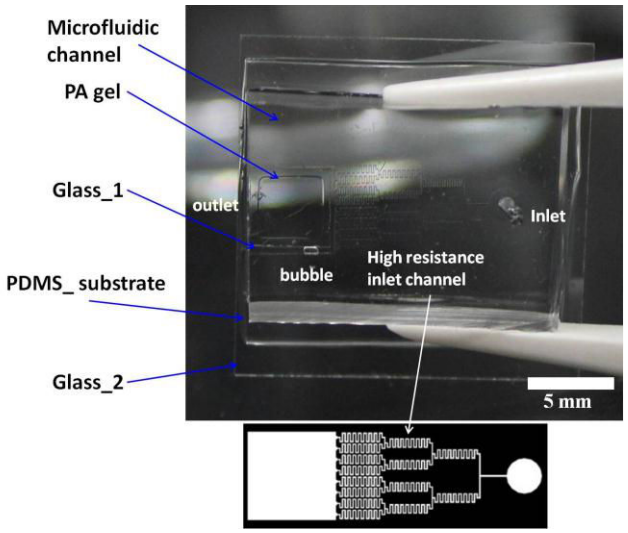

Figure 4. A photograph of the assembled device and the mask design of the microfluidic channels.

IV. Results and Discussion

A. The integrated PA gel-microfluidic device

The device is shown in Fig. 4, and a picture of the photomask is also shown below it to demonstrate the microfluidic channel design more clearly. The long inlet channels are designed to increase the hydraulic resistance in these channels, and that high resistance can increase the flow stability in the source side of the cell culture chamber. This will be important to have uniform concentrations along the direction perpendicular to the input flow. The whole device is transparent, which is an advantage for cell-research users because this will be compatible to their daily instrument, for example, the phase contrast microscope. However, the thickness (1 mm) of the PDMS substrate will be an issue if the user wants to study cell organelles or cytoskeletons. The possible solution being tested for this issue is replacing the groove with a hole and decreasing the thickness of the PDMS substrate simultaneously. Thus, we could build the PA gel directly on Glass_2. Air bubbles appear sometimes in the device during injecting deionized water into the cell culture chamber by connecting the inlet to the syringe. However, it does not affect the performance of the device since the bubbles will disappear easily when we immerse it in the deionized water overnight. The device can be kept in sterilized deionized-water for over one week before the functionalization process of the PA gel.

B. Simulation of the sucrose concentration gradient

The simulation results are shown in Fig. 5. There are eight inlet ports to the cell culture chamber, and these eight point sources form a line source to supply the solute to diffuse into the cell culture chamber. The line source will be more appropriate to generate uniform concentration gradient along different position of y-axis. The arrow surface also shows the velocity field is uniform in the cell culture chamber. The data suggests that the device needs six to ten hours to have a stable gradient (Fig. 5, b) by computing the gradients change along the white arrow (Fig. 5, a) in a time dependent study. From these simulations, we also noted that the highest concentration in the source side of cell culture chamber is slightly lower (e.g. 190 mol/m^3) than the inflow setting (e.g. 200 mol/m^3). In fact, when the position of the inlet was set much far from the cell culture chamber, this concentration drop between the inlet and source side would be more significant (e.g. 200 mol/m^3 to 65 mol/m^3, data not shown). That can be an important reference to decide what the concentration would be in the inlet to generate the gradients we need in the cell culture chamber.

There is no significant difference in the concentration distribution between free flow area and porous media when we set the thickness of the two layers are equal to 100 µm (Fig. 5, c). However, the flow velocity profiles in these two areas are quite different caused by the low permeability (10^{-14} m^2) of the PA gel. The highest flow velocity in the free flow area is about 0.15 µm/sec (Fig. 5, d), but it is almost zero in the porous media. It might explain that the fluorophore still existed in the PA gel after wash the whole cell culture chamber with deionized water several times. The velocity profile is similar with other simulation work for free flow-porous media (COMSOL model gallery, Model ID: 170).

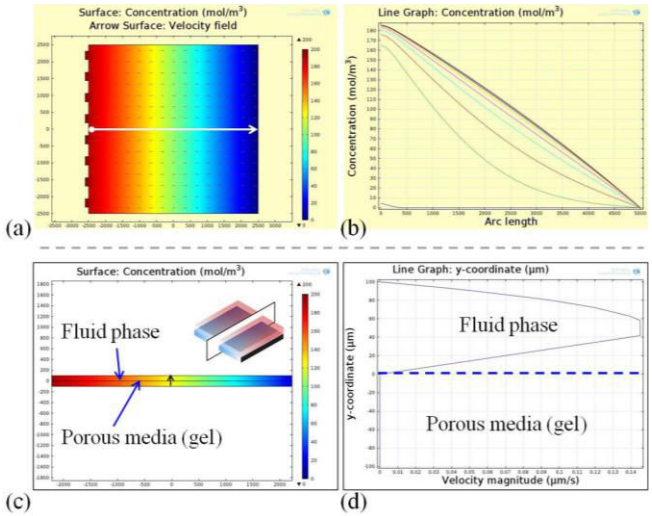

Figure 5. Simulation of concentration gradient and data plots.

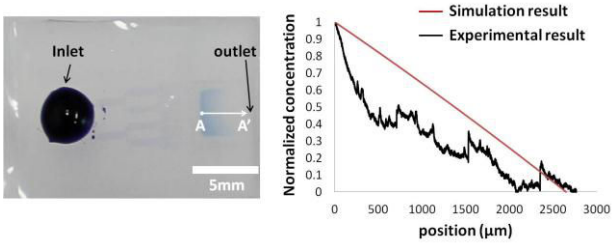

Figure 6. A photograph of the trypan blue concentration gradient generated by the device. The data plot (right) describes the concentration gradient from A to A' (left), which is the width of the PA gel.

C. Trypan blue concentration gradient

The color gradient of trypan blue (Mw＝872.88 Da) was used to simulate the sucrose (Mw＝342.3 Da) gradient in the cell culture chamber .The concentration gradient of trypan

blue generated by our device is shown in Fig. 6. This color gradient existed for over than 36 hours. The concentration gradient upon the PA gel (A→A') is plotted on the right figure. Despite the experimental result dose not fit much to the simulation result, they show the same trend.

D. Cell culture in the integrated PA gel-microfluidic device

We have cultured C2C12 cells in four different devices (Fig. 7). Three of four are successful, and the one (device 4) failed to culture cells might have a non-functionalized PA gel. This assumption is lead by two observations. First, there are cells attached on Glass_1 in device 4, thus the ECM protein and other conditions (pH, temparature…etc.) must function appropriately. Second, even the rough surface in device 3 doesn't affect cell culture much. The cause of failed ETC-fibronectin reaction should come from inappropriate bonding between microfluidic channel and PDMS substrate. During the polymerization step of PA gel (Fig. 3, b), a coverslip silanized with dichlorodimethylsilane (DCDMS) was used to smooth the gel. The DCDMS might transfer from the coverslip to the surface of PDMS substrate and make bonding failed in the containminated area. When injecting the reactants into the failed-bonding device with a syringe, the flow will leak out the device through these failed-bonding regions, which are lower hydraulic resistance channels. This leakage can also be found when the failed-bonding device was tested with fluorescent dye. If the DCDMS transfer issue is addressed properly, the device is easy to perform cell culture with only slightly changes of functionalization of PA gel and cell culture protocol.

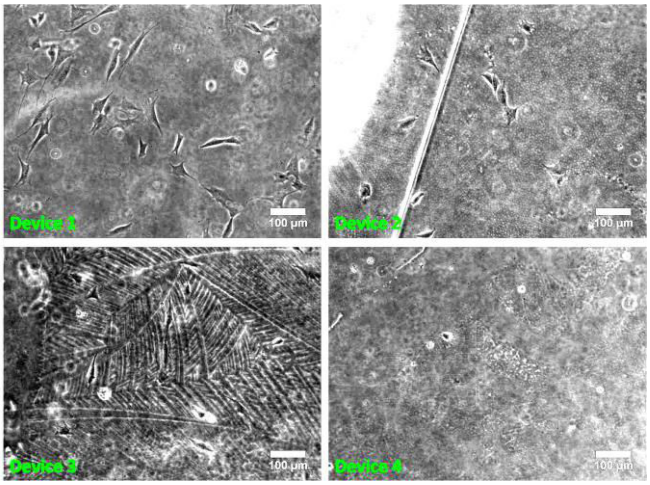

Figure 7. Cell culture after seeding for 24 hours in the devices. Only device 4 failed to have a normal cell culture result.

V. CONCLUSION

The device presented in this study might be the first one to embed a gradient-compliant PA gel in a microfluidic system, and this device has the potential to be used to study cell migration under two different stimulations which is more close to the in vivo system. The first stimulation is stiffness gradient, and the second one can be osmotic gradients or theoretically, chemical gradients if the solute is replaced with chemoacttractants, thus making the device more flexible to be adopted for other studies.

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