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# Volume Change Measurements of Cancer Cells in a Microfluidics Platform

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Volume Change Measurements of Cancer Cells in A Microfluidics Platform

by

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# ABSTRACT

Understanding the function of aquaporins in transcellular transport is vital for homeostasis, pathophysiology, and translational potential in AQP-based diagnostics and therapeutics. Microfluidics device has the potential to evaluate the function of aquaporins with precise control and manipulate micro-scale fluid due to good optical quality and short processing distance. This work has two parts: cell culture in a microfluidic chip to characterize aquaporins' function and visualization of single cell volume change under osmotic pressure shock in a homemade imaging dish. For the first part, we established a microfluidics platform for future modification and measurement of the dynamic volume change of biological cells. Then, we used the layer-by-layer method to microfabricate the microfluidics device made of acrylic plates and pressure-sensitive adhesive. It is easier to make simple-patterned microchannels and provide better optical quality for bioimaging by attaching a thin microscope coverslip. MDA-MB-231 breast cancer cell line was used for this study. When culturing cells in microfluidics devices, we improved the coating method to ensure the cells attach to the microchannel wall. Meanwhile, reducing the flow of culture medium inside the chamber can improve cell growth in an incubator. Our results showed that MDA-MB-231 could grow well in our microfluidics platform.

For the second part, we used a microinjection system to study the cell volume change caused by water flux through the plasma membrane of a single cell under osmotic pressure shock. The dynamics of cell volume change were visualized and measured using a microscope with an oil immersion objective and ImageJ. The change in cell volume was

monitored and measured in our system with a corresponding reduction of environmental osmotic pressure. This research confers new opportunities when utilized in monitoring and measuring transcellular transport phenomena of a single cell for in-vitro experiments.

Keywords: microfluidics device, MDA-MB-231, cell volume change, osmotic pressure

# TABLE OF CONTENTS

# LIST OF FIGURES

# LIST OF ABBREVIATIONS

AQP..... Aquaporin

DMEM .....Dulbecco's Modified Eagle Medium

FBS .....Fetal Bovine Serum

PDMS.....Polydimethylsiloxane

PMMA .....Polymethyl Methacrylate

# Chapter 1

# Introduction

## 1.1 Background about aquaporins

Aquaporins (AQPs) are a family of small, pore-shaped, water-transport proteins in the lipid membrane [1]. They have more than 20 members and are distributed in various tissues. AQPs also widely exist in human cells, maintain the homeostasis of the internal environment, and participate in many physiological processes.

Many studies showed that AQPs were closely associated with biological cancer functions, one of the leading metastases causes [2]. The mechanism of AQP1 in promoting tumor proliferation includes three aspects: cell cycle, regulating cell volume, and cell signal transduction pathway by interacting with other membrane proteins or transcription factors [3]. AQPs are also related to angiogenesis and tumor diffusion because they facilitate cancer cell migration [4]. The expression of AQP 1 in cancer cells increases the number of cells while increasing the osmotic gradient across the cell membrane, which may promote angiogenesis by promoting the release of angiogenic factors and cytokines. Hence, cancer cells can exude from blood vessels and invade human tissues.

Cell proliferation is closely related to cell volume changes with the help of AQPs. In the cell cycle process, the cell volume increases continuously with protein synthesis and DNA replication [5]. The study found that the overexpression of AQP1 can increase the permeability of hydrogen peroxide and regulate the expression of protein related to cell proliferation. Cancer cell volume is more significant than a normal cell, suggesting that AQP1 may promote tumor proliferation by increasing cell volume. In addition to changing cell volume and adjusting cell membrane potential, AQP1 can activate downstream signal transduction pathways by interacting with other membrane proteins or transcription factors to promote tumor progression. Studies have shown that the hypoxic environment can also promote the expression of AQP1 [6]. The up-regulation of AQP1 can change the cell volume, adjust the cell membrane potential and activate a series of downstream signal pathways such as Wnt and FAK, thus affecting the process of cell proliferation and promoting the progress of the tumor.

Above all, aquaporins play an essential role in cell growth. Therefore, aquaporins can be considered potential targets for developing new cancer therapies. In addition, inventing new drugs that can selectively activate or deactivate specific aquaporins may be a new treatment for cancer, hypertension, heart failure, etc. So, it is necessary to design a device to study the water flux at the single-cell level. The microfluidic device could provide an opportunity to generate the water flux through the water channels and find a method to measure the flux through a single cell and its response to the change in stimuli [7].

## 1.2 Background about microfluidic devices

Microfluidics devices were extensively studied for observing in-vitro experiments [8], such as biomedical analysis, pharmacokinetics, and cell performances. Microfluidics devices usually have the characteristics of simplicity, small hydraulic diameter, and short processing distance, which facilitate a wide range of biomedical experiments [9]. Meanwhile, microfluidics devices can effectively provide a laminar and fully developed flow for a biological experiment since these devices usually have a higher surface-area-to-volume ratio, better heat transfer rates, and fewer extraneous variables than other devices [10]. Furthermore, microfluidics devices have been identified as a 3D culturing system because of their ability to supply convective nutrients for cells [11]. The field of microfluidic devices is rapidly advancing and will have a bright future.

In the regime of microfluidics devices, the cell volume change is an essential concern for researchers since this technology presents innovative solutions to diagnostic and clinical cancer challenges [12]. Various publications have been about cell volume change by changing the environment of a microchannel with different osmotic pressure. Furthermore, stimulating the background of blood vessels is also an essential part of lab-on-a-chip, which allows people to investigate pathophysiological situations of cancer cells and metastasis [13]. In short, this thesis aims to measure cancer cell MDA-MB-231 performances in microfluidic devices and the design method and possible conditions that may affect the environment in microfluidic devices.

Microchannel technologies usually include replica molding, soft lithography, and 3D bioprinting [14]. The replica molding was used to fabricate the 2D geometry of microchannels complementary to molds patterned by photolithography [15]. In addition, replica molding and soft lithography can contribute to the final microchannel by stacking multiple matrix layers of complementary parts.

In recent years, photolithographic patterning techniques have become essential for making a microfluidic device and modifying micro-scale biochemical properties [16]. Photolithographic polymerization, or photolithography, describes a process used in microfabrication to pattern parts on a microfluidic system. It uses UV light to transfer a geometric pattern from a photomask to a photosensitive chemical photoresist on the microfluidic system. Therefore, some researcher considers it UV lithography [17]. This method could enable the deposition of new material in the desired pattern upon the material underneath the photoresist.

Furthermore, photodegrade crosslinks can form channels through direct laser irradiation and build microarchitectures via sequential additive photopatterning [18]. Applying these methods is helpful to pattern 2D fluidic structures. The multi-layer stacking technique, in which multiple layers of 2D microfluidic networks are assembled in a vertical stack with vertical connecting vias, has been adopted because it leverages the high-resolution capabilities of lithographic 2D microfluidics, requires no additional hardware, and is relatively straightforward [19]. Meanwhile, the pattern determines the channel geometry in 2D, and the layer thickness, number of layers, and size of the vertical

connecting pipe govern the channel density in the third dimension. Thus, the formation of structures with high channel density in 2D can be easily achieved using the appropriate pattern. However, realizing high channel densities in all three dimensions requires thin layers with extremely stringent layer-to-layer alignment techniques [20]. The microfluidic device could be made by this rapid photolithographic technique—the photolithography masks bearing the channel design printed on high-resolution films [21]. The hostile masters were created from a photo-patternable epoxy spin-coated onto silicon wafers and exposed to UV light through the film negative of the desired channel size. The silicon was then etched with inverse structures of the microfluidic channels.

3D printing is another vital method to fabricate the microfluidic channel. Some microfabrication methods of fabricating microfluidic devices are unsuitable for handling hydrogel, so 3D printing helps solve the problem [22]. 3D printing can produce an entire microfluidic device by polymer jet printing, fused deposition modeling, and sacrificial molding techniques [23]. To fabricate a microfluidic device, PDMS is poured onto a master [24]. After obtaining the channel mold, the PDMS replicating and ABS dissolving procedures were sequentially conducted to achieve a microfluidic chip. After curing, the rubber must be carefully peeled off from the master and chemically bonded to another surface after activation with oxygen plasma or using chemical solutions.

## 1.3 PMMA microfluidics devices design and fabrication

Designing a microfluidic device with good optical quality is the first step for measuring cancer cells' performance. As Tsvirkun mentioned in his paper [25], a microfluidic device consists of a microchannel network, a microscope, and one or several cell lines. Microchannels should be made from transparent materials to facilitate using a high-resolution microscope. What's more, to stimulate the environment of organisms in-vitro or overcome the limitation of material, some chemical agents like hydrogel or silicone elastomer may consider as a part of a microfluidic device [26].

From the above understanding of microfluidic chips, selecting chip manufacturing materials is essential and should meet the following principles [27]: The material has good chemical and biocompatibility and cannot react. Otherwise, it will cause damage to the chip; The material has electrical insulation and heat dissipation for better operation; The fabric has little or no interference to the detection signal; The material is easy to obtain, and the production procedure is simple; The material has good modifiability and can produce electroosmosis or immobilize biological macromolecules. Many microfluidics chips aim at studying laminar hydrodynamic flow.

This research focuses on trying a new method to culture cells in a steadier platform rather than Polydimethylsiloxane (PDMS) platform and applying microfluidics devices to measure cancer cell volume change [28]. PDMS is considered a common material that can be used to build microfluidic devices. However, since PDMS has an inherent hydrophobic nature, the usage of PDMS was limited in many applications [29]. Therefore, many

microfluidics systems feature rapid and gentle processing conditions to directly afford cell encapsulation within the hydrogel or transform the hydrophobic to hydrophilic. Instead of PDMS, we choose Poly- (methyl methacrylate) (PMMA) as the substrate since PMMA has better scratch resistance. PMMA is a transparent, rigid, and recycled plastic material, which is also known as acrylic in the manufacturing and scientific fields [30]. Passive microfluidics devices are geometry-dependent. Therefore, the surface quality of PMMA can help maintain the shape of the microfluidics device and keep the experiments repeatable. We choose double-sided tapes to stick the PMMA plate and cover slip together to better shape the microfluidics devices' pattern. Double-sided tape has a more solid structure than all kinds of glue. Meanwhile, we need to curve the microchannel pattern on the linkage. Double-sided tape's transparency and soft character make it a cheap and better material to form a chamber of a microfluidics device.

According to researchers' experiments, the main structure of a microfluidics device is divided into upper and lower layers of the film base [31]. These film bases need good optical quality so that detective devices can receive clear signals. PDMS and PMMA can be chosen as the material [32]. An entire microfluidics device may include structural units such as sample inlets and detection windows. Peripheral equipment includes peristaltic pumps, micro-injection pumps, temperature control systems, and detection components such as ultraviolet, fluorescence, electrochemistry, and chromatography. Therefore, flexible plasticity is also needed to fulfill the need of the experiments. Microchannels should be made from transparent materials to facilitate using a high-resolution microscope.

## 1.4 Surface coating of microchannel

Different coating methods have been applied in past research to transform the surface properties of PMMA for the cell experiments, such as plasma oxidation, thermal aging, and chemical coating [33]. For gelatin coating on a microfluidic system, covalent bonds polymerize monomer units into polymer chains, crosslink the polymer chains into a polymer network, and interlink the polymer network to the microfluidic system [34]. Monomer units and coupling agents copolymerize into polymer chains but do not crosslink into a network, resulting in an aqueous solution. The solution may contain other compounds for various functions [35]. Functional groups complementary to the coupling agents are imparted onto the surface of a microfluidic system. After the solution contacts with another substrate, the coupling agents react with each other to crosslink the polymer chains into a network and react with the complementary functional groups to interlink the polymer network to the microfluidic system [36]. For example, the surface of treated PDMS contains hydroxyl groups. It can react with the silanol groups in the hydrogel paint to form siloxane interlinks between the elastomer and the hydrogel [37].

## 1.5 The new method to test water permeability of aquaporin

Nowadays, three methods can measure the water permeability in microchannels: sampling weighing, observing changes in mass or volume versus time, and Nuclear Magnetic Resonance [38]. For example, total internal reflection fluorescence microscopy could measure osmotic water permeability in adherent cells. Cells are loaded with a

membrane impermeant volume marker. A thin layer of the cytosol is illuminated by a laser beam directed through a glass prism at a subcritical illumination angle. As the cell shrinks in response to an osmotic gradient, fluorophore concentration in the illuminated region increases, increasing the detected signal. However, there is a more accurate instrument to test water permeability.

A powerful velocimetry, laser-induced fluorescence photobleaching anemometry (LIFPA) has been developed [39], which uses a molecular tracer with a high temporal and spatial resolution to measure the velocity profile in micro capillaries, and it could be used to measure the water flux of aquaporin. In LIFPA, a molecular tracer of fluorescence dye and the photobleaching effect are applied as a transducer to measure the flow velocity. The velocity is calculated by measuring fluorescence with a calibration relationship between the speed and fluorescence. The temporal resolution has been achieved to 5 μs, and it appears that this method has the potential to improve the temporal resolution further. LIFPA is based on the photobleaching of a fluorescent dye for microfluidic devices. The residence time of the fluorescent dye in a laser beam depends on the flow velocity. It approximately corresponds to the decaying time of the photobleaching of the dye in the laser beam. Therefore, the residence time is inversely proportional to the flow velocity. The new method can quickly measure the velocity and is easy to use. It is demonstrated for both pressure-driven flow and electroosmotic flow.

Besides the help of LIFPA, Stimulated Emission Depletion Microscopy (STED) could also be used to focus the laser beam to overcome the diffraction limit. STED microscopy

localize fluorescence at each focal spot using two laser pulses [40]. One pulse is used to excite a fluorophore to its fluorescent state. The other can de-excite any fluorophores surrounding the excitation focal spot, so the focal spot is raster scanned across the sample and generate the needed image.

With the help of LIFPA and STED, aquaporin's water flux could be measured. However, this method also has a big problem. It has to have a relatively high velocity, or its signal will weaken. Carbonic Anhydrase II and Chelerythrine are two kinds of compounds that could increase the water flux of aquaporins [41]. Usually, some material could upregulate the aquaporin expression to increase the water. Still, both materials could increase aquaporin's water flux within a few minutes by changing the structure of aquaporin. And they would help measure the water flux accurately. Using these two kinds of materials, we could compare the result of water transport across the limiting membrane of a cell or sealed membrane vesicle or liposome measured from the time course of cell volume change in response to an osmotic challenge. So we could know the accuracy of the combination of the two devices.

## 1.6 Measurement of cancer cell behaviors in microfluidic devices

### 1.6.1 Flow velocity

Velocity is essential when observing cancer cell behaviors, and researchers often use microfluidic particle image velocimetry (micro-PIV) to measure the velocity. Micro-PIV is a quantitative, non-intrusive technique for field measurement at microfluidic channels,

and it makes the highly accurate measurement for micro-and nano-liter flow rate realized [42]. The flow rate can be approached on one cross-section for the microchannel without a branch. Flow rate measurements of every branch can also be achieved for the microchannels with branches, which is almost impossible for conventional methods. Regarding some exceptional cases, such as flows in microtubes or rectangular microchannels, the flow rates can be approached by the velocity distribution along the radius or on the median plane. These velocity distributions are governed by section-averaged velocity. In this work, the flow rates of water in square microdots are achieved by the velocity distributions on median planes by employing a micro-PIV system. The technique of micro-PIV is based on the statistical analysis for displacements of tracking particle groups in several interrogate windows of particle images and calculates the flow rate by the quotient of displacement and time delay [43]. A micro-PIV system includes the following indispensable components: fluorescent tracking particles seeded in flow fields, the pulse laser system for illuminating the tracking particles two times in the time delay, a microscopic imaging system including a CCD camera with susceptible chip and microscopic lenses system for acquiring the two frames of particle images, synchronizer to control the laser and CCD camera working synchronously as well as a software system for particle image analysis, displacement calculations for gaining of the velocity distributions of flow field [44]. As Nath mentioned in his paper, The velocity of cells moved through the microchannel at high speed can also be captured by a high-speed camera at 50000–60000 fps using 20X objective in a microscope [45].

### 1.6.2 Using electrical filed to test cells' behavior

The roles of electrical stimulation (ES) in regulating cancer cell behaviors in microfluidic devices have been gradually recognized [46]. Microfluidics-based ES methods have been explored to electrically stimulate cell clusters or single cells without attaching/growing cells on the electrodes [47]. When a liquid is in a microfluidic device within an electric field, several phenomena might occur depending on the type of liquids, liquid-surface interface, and nature of electric fields, generally known as electrokinetic.

Electroosmosis is the motion of ionized liquid for stationary charged or polarized surfaces in the presence of an applied electric field [48]. Depending on the nature of the applied electric field and device configuration, electroosmosis can be classified as DC electroosmosis, time-periodic electroosmosis, AC electroosmosis, and induced charge electroosmosis. The electric field can also transport micron and submicron-sized particles immersed in a fluid if these particles are charged [49]. Their motion is governed by electrophoresis or combined electrophoresis and electroosmosis.

## 1.7 Stimulus

### 1.7.1 Chelerythrine

Inhibition of PKC (a known regulator of glioma cell invasion) activity with Chelerythrine could reduce AQP4 phosphorylation, enhance water permeability, and significantly improve water permeability enhances tumor invasion so that it could increase

the water flux of AQPs [50].

### 1.7.2 Carbonic anhydrase II

Transepithelial sodium movement across the proximal tubule creates a concentration gradient that drives water reabsorption [51]. Sodium moves across the proximal tubular cell's apical plasma membrane in exchange for a proton's efflux, mediated by the sodium proton exchanger isoform 3 (NHE3). The catalysis of water and CO2 into H+ and HCO3− via cytosolic carbonic anhydrase II (CAII) participates in the reabsorption of water and sodium by providing H+ for the exchange reaction. So Carbonic Anhydrase-II could indirectly increase the water flux of aquaporin.

According to the research mentioned, we could conclude the following data: When the mannitol gradient goes up, the water flux of aquaporins becomes larger; When using stimuli to the membrane, the water flux of aquaporins becomes larger. Therefore, Chelerythrine has a better effect than CA II—water flux, which is one of the indexes that could show water permeability.

# Chapter 2

# Materials and Methods

## 2.1 Culture of MDA-MB-231

The breast adenocarcinoma cell line MDA-MB-231 was obtained from the American Type Culture Collection (ATCC). The cells were maintained in Dulbecco's Modified Eagle Medium (DMEM) (Corning) supplemented with 10% Fetal Bovine Serum (FBS) (Sigma-Aldrich), mixed with 1% Penicillin-Streptomycin (ATCC) in a humidified incubator (37°C, 5% CO2). In addition, the cells were passaged with standard 0.25% trypsin-EDTA (Corning) when the cell confluency reached 75%-90%. Cancer cell lines MCF7 were obtained from Wuhan Elabscience Biotechnology Corporation and were cultured in DMEM supplemented with 10% FBS. The cells were maintained in an incubator with a constantly humidified atmosphere and 5% CO2 at 37 °C.

## 2.2 Surface coating of coverslips

Before shaping the microfluidics device, coverslips must be well-prepared to ensure the cells can firmly attach to the selected area.

Add 2% gelatin solution into a glass bottom dish or plate at 5 ug/cm2. Before using the gelatin solution to coat, it is necessary sterilizing the gelatin solution autoclaving at 121 ℃, 15 psi for 30 minutes, since the cell culture environment needs a strict environment without any bacteria or contaminations. If cells are cultured only on the glass surface, then only the glass surface must be covered with gelatin solution. The coverslip is fixed on a spin coater by tape. After its spin speed becomes stable, drip 0.05 mL solution onto it, and spin it for around 5 mins. So the gelatin could form a transparent film and coat the coverslip. Use UV light to disinfect the coverslip for 1 hour. Then, rinse three times with PBS or serum-free media. Gelatin-coated glass bottom dish or glass bottom plate can be kept at 4℃ for about a month. If so, it needs to be disinfected again by UV light before using it. Note that coating on a glass surface is less stable than coating on plastic tissue culture dishes or plates. Thus, coating the coverslip before cell culture is recommended.

2.3 Shape the microfluidics devices

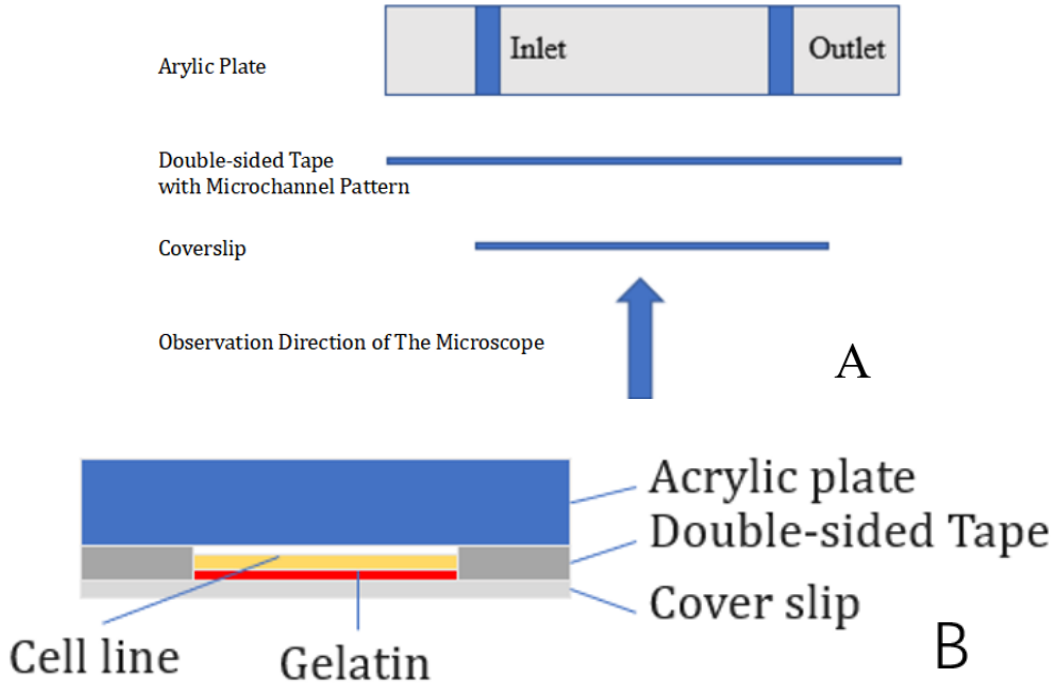


Figure 2.1 The essential components of a chip. A: Side view, B: Front view

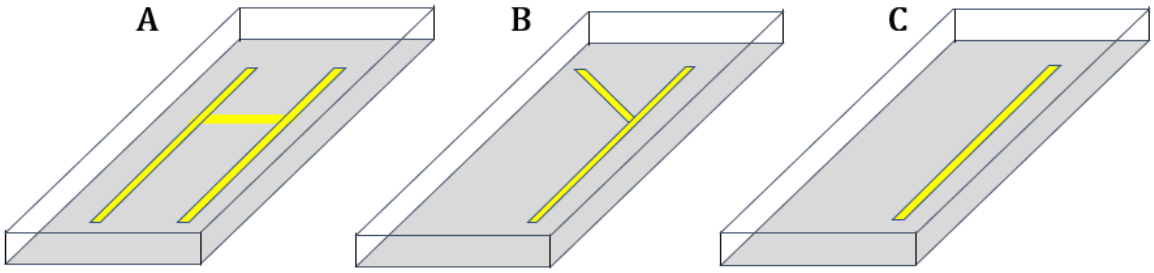


Figure 2.2 Different designs of microchannel, top view.
A: H-type microchannel B: Y-type microchannel. C: I-type microchannel.

Our microfluidics devices include acrylic plates, double-sided tapes with a microchannel pattern, and gelatin-coated coverslip (Figure 2.1). According to the purpose of our experiments, we could shape different types of the microchannel. For example, an

H-type microchannel can do the paralleled experiment; A Y-type microchannel can study the influences of diffusion to the cells; an I-type microchannel can set up a control group for the experiment. (Figure 2.2).

To prepare the microfluidics device, use a drill table to shape inlets and outlets on an acrylic plate. Before putting the acrylic plate on a drill table, mark the locations on the plate based on your design. When moving the drill, do it slowly and stably to make sure the shape of the hole is regular. After drilling through the holes, repeat to drill in the same place two or three times to ensure no acrylic residue in the hole. Cut the microchannel pattern on a stiff scaffold since it is essential to stack two or three pins to fix the tapes. Mark the location of your inlets and outlets on the tape based on the microchannel design. Mark the place location of your inlets and outlets on the tape. Fix the tape on the wood scaffold and cut off the surplus tape. Dry the glass slide, and immobilize the glass on a flat. Apply the double-sided tape with the microchannel pattern carefully to the acrylic plate. Remove the cover of the tape and put the cover glass on it. Put a heavy object gently on top to apply pressure for a few minutes. Cut off some PMMA tubes and glue them on the inlets and outlets of microchannels with Epoxy glue as the reservoir to store medium and protect cell lines. Put a heavy object gently on top to apply pressure and leave for a few hours. Cut 25mm acrylic pipe and glue it on the inlet and outlet of the microchannel as the reservoirs. Water-seal it for 24 hours to ensure the microchannel is stable (Figure 2.3). Then, test if the microchannel fits the design size (Figure 2.4).

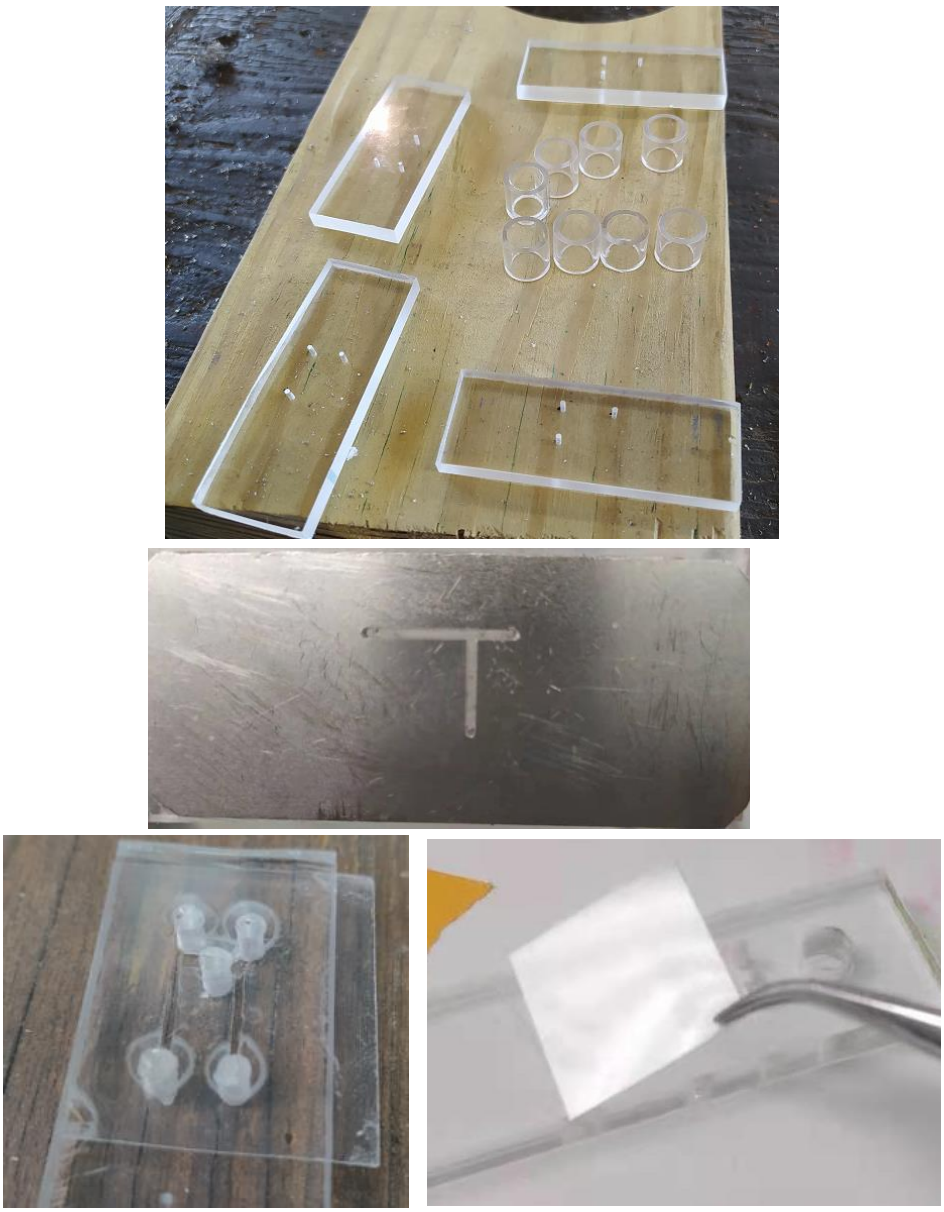

Figure 2.3 Steps for fabricating microchannels.
A: Prototype of an acrylic plate, B: Model of the microchannel,
C: A sample of an H-type microchannel pattern, D: Seal it with a coverslip.

Figure 2.4: Completed microfluidics devices

2.4 Preparing single-cell suspension

Firstly, we remove all spent medium from cell culture vessels and evenly distribute the Trypsin over the entire cell layer. Then, we incubate the cells in their culturing environment (5% CO2 at 37 °C) for 5 minutes. Once we observed the cell layer has detached, dilute out the passaging reagent with a prewarmed medium to stop the digesting reaction. After we centrifuge the cells for 5 minutes and repeat the harvest of the MCF-7 and MDA-MB-231, we may get the single-cell suspension.

2.5 Pre-experiment on the Petri dishes

Before culturing the cell line in the microfluidics devices, we want to ensure that the cell can successfully grow in the same cover glass without the PMMA plate and test its volume change rate (Figure 2.5). Therefore, we used 0.1% gelatin to coat the same cover glass and glue it at the bottom of a petri dish for three days.

Figure 2.5: Injection system for Petri dishes experiments

## 2.6 Changing osmotic pressure in a Petri dish

After the cell is well prepared, increasing osmotic pressure is controlled by adding 0.25 μL sterile DI water through the InjectMan NI2 system (Eppendorf). To reduce the environmental noise, we should empty the medium before injecting the DI water.

## 2.7 Using ImageJ to analysis data

After observing the cells' area change under the microscope and recording videos, use the freehand selection tool to draw around the edge of each cell in the image. Then, according to the scale of the injection system, go to "Measure" and calculate the area of cells. Repeat this process for each cell in the image for several selected time points and draw a curve. And we could estimate the volume-changing ratio of cells by comparing the initial area.

2.8 Culturing cells in the microchannel

MDA-MB-231 is dissociated and resuspended by centrifuging machine for cell seeding into the gelatin crosslinked chips. Following this, the suspension is loaded into the fluidic channel, and the MDA-MB-231 is allowed to grow until the needed confluency. Finally, cover the reservoir with caps to reduce the evaporation of the medium (Figure 2.6).

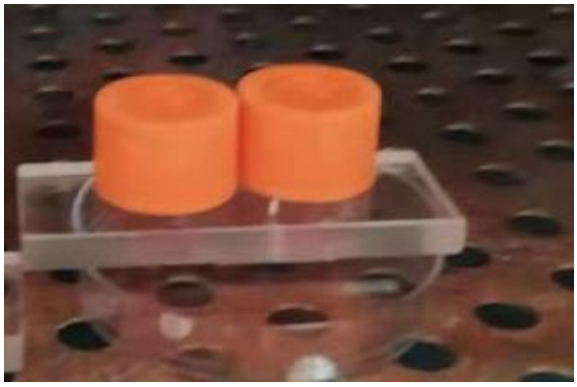

Figure 2.6: Cover the reservoir with caps

# Chapter 3

# Result

## 3.1 Cell volume changing phenomenon in the Petri dish

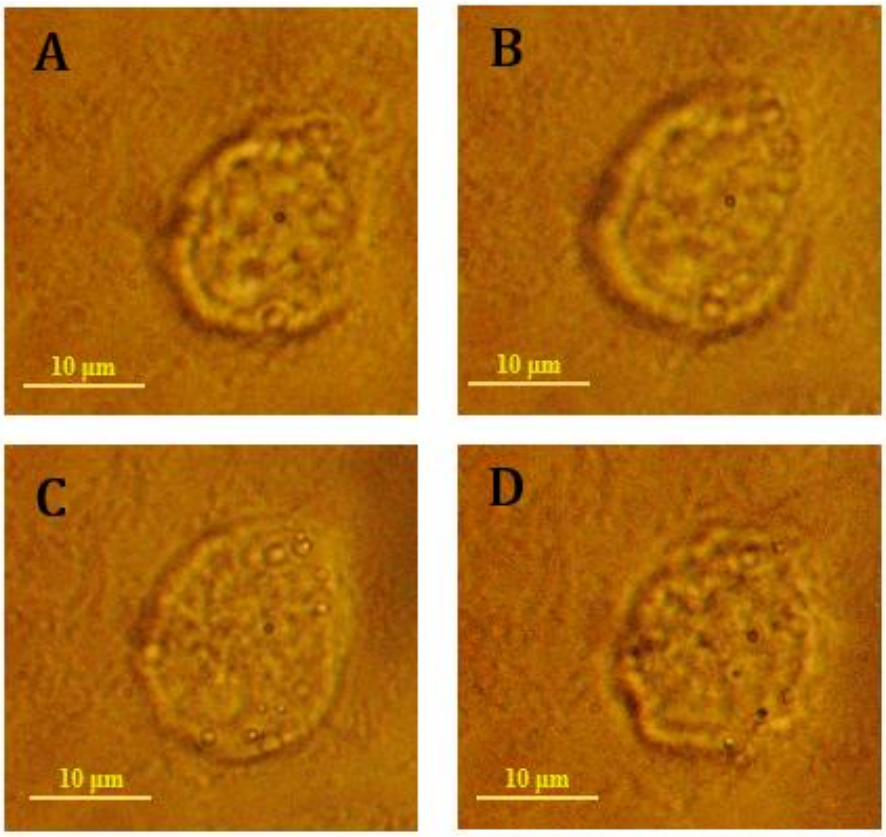


Figure 3.1 Attached MDA-MB-231 cells that have a regulation phenomenon.

A: Attached cell area before injecting DI water.

B: After 30 s. C: After 60 s. D: After 90 s.

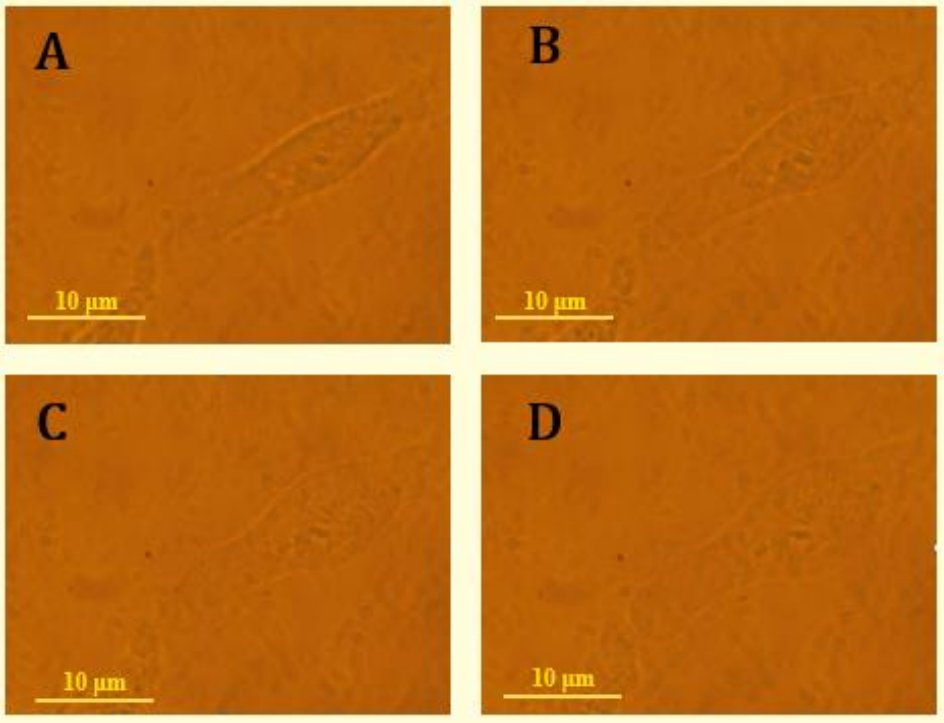


Figure 3.2 Attached MDA-MB-231 cells with no regulation phenomenon.

A: Attached cell area before injecting DI water.

B: After 30 s. C: After 60 s. D: After 90 s.

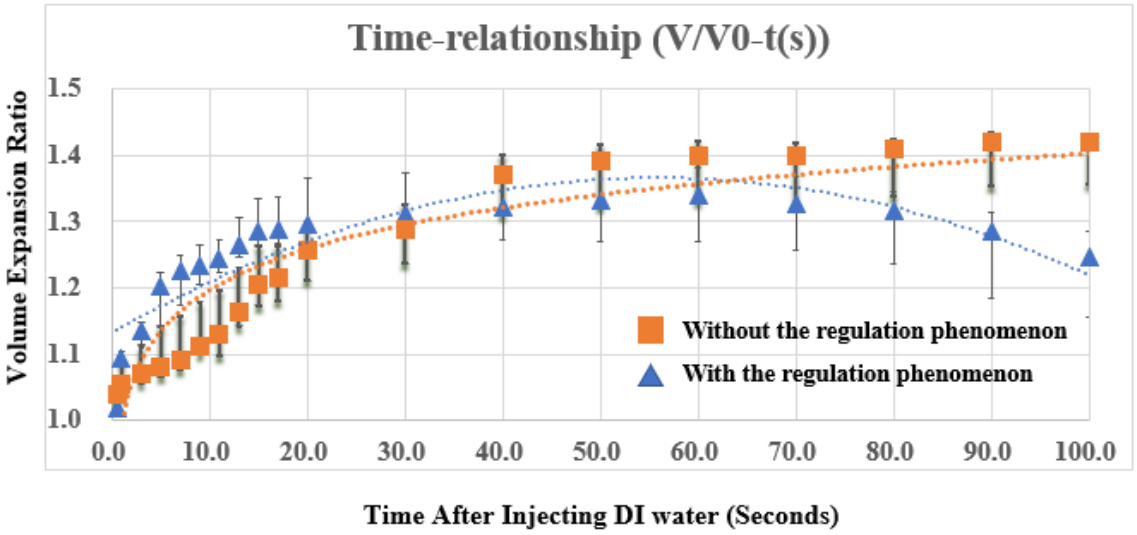


Figure 3.3 Volume change of attached MDA-MB-231. The blue line is the one with the regulation phenomenon happening. The orange line is without the regulation phenomenon.

Our experiments showed that even in the same environment, the cell might have two results after injecting DI water. One is the cell starts shrinking after it swelled to a certain degree (Figure 3.1 ). A cell can reduce its volume after swelling in a hypotonic environment by synthesizing and activating osmolytes [52], which allows them to interact with and remove water from the cell. After the cells start swelling, the cell membrane becomes stretched, which generates a force that opposes further swelling [53]. This phenomenon is known as the membrane tension or surface tension, and it increases as the cell membrane expands. For those well-attached cells, the membrane tension becomes strong enough to balance the osmotic pressure gradient with the help of osmolytes, and the cell stops swelling. Another possible reason is the activation of ion transporters and channels. These transporters and channels allow ions, such as potassium and chloride, to move out of the

cell. It results in a decrease in the intracellular osmotic pressure and subsequent release of water from the cell. This process, known as regulatory volume decrease (RVD), helps to restore the intracellular solute concentration [54]. Therefore, the osmotic pressure changes fast initially, but the regulation system generally takes effect and reaches a balance afterward. Therefore, the curve of attached cells is logarithmic (Figure 3.3).

Meanwhile, those cells that don't have a shrinking phenomenon would keep swelling and start floating, and finally, their cell membrane would break (Figure 3.2). So, two different phenomena in one environment depend on the attachment degree of cells and biological activity. Cells require a substrate to attach to in order to maintain viability and perform their functions [55]. When cells are in suspension, they are exposed to stresses that can reduce their viability and alter their behavior. Researchers can create a stable microenvironment that promotes cell viability and function by attaching the cells to the microchannel's surface. Lack of attachment may cause the cells to have less contact surface with the coverslip and be more easily affected by the environment. And less cell activity may cause lower activity of ion channels and other regulatory mechanisms.

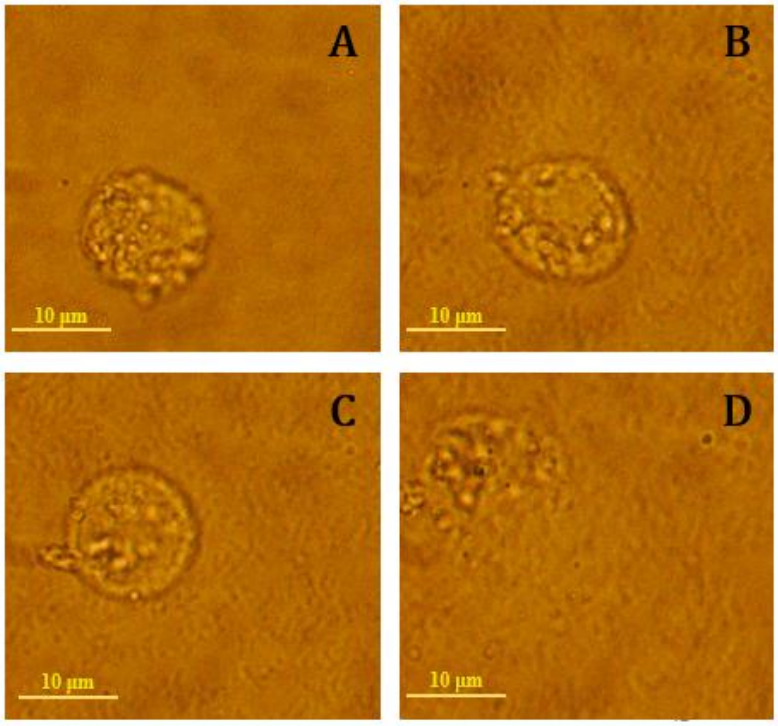


Figure 3.4 Floating MDA-MB-231 cells

A: Attached cell area before injecting DI water.

B: After 30 s. C: After 60 s. D: After 90 s.

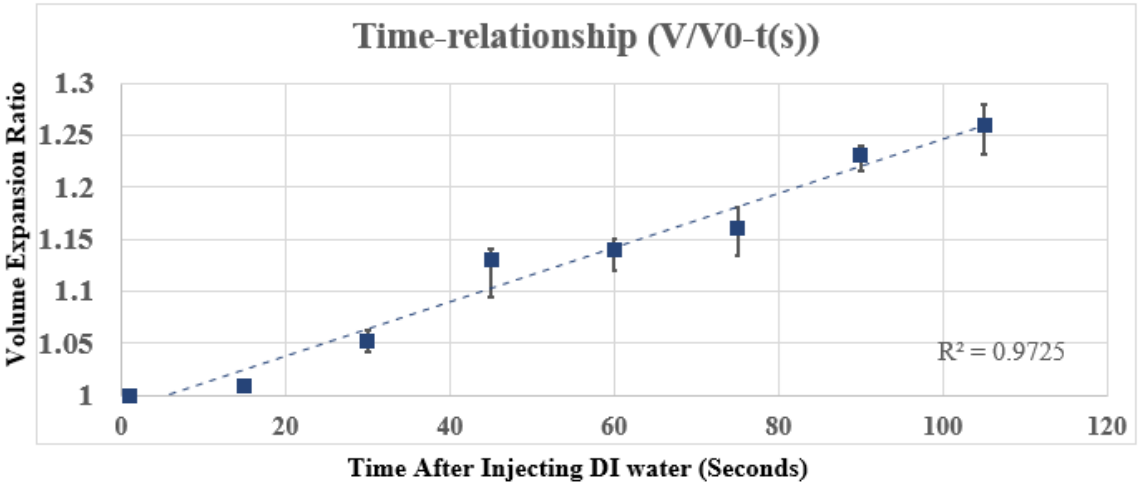


Figure 3.5 Volume change of floating MDA-MB-231.

The volume change for the floating cell is almost linear since the osmotic pressure gradient across the cell membrane is in all directions (Figure 3.4, Figure 3.5). Meanwhile, the attached cells have a large area that would not contact the medium but attach to the gelatin-coated coverslip, so the water influx is relatively restricted. In a hypotonic environment, the concentration of solutes outside the cell is lower than the concentration inside the cell. It creates a concentration gradient that drives water to move into the cell. This influx of water causes the cell to swell and increase in volume. The strength of membrane tension is more evident for the well-attached cells. Therefore, the attachment degree of cells could be a pivotal effect in deciding whether a cell would have a regulation phenomenon.

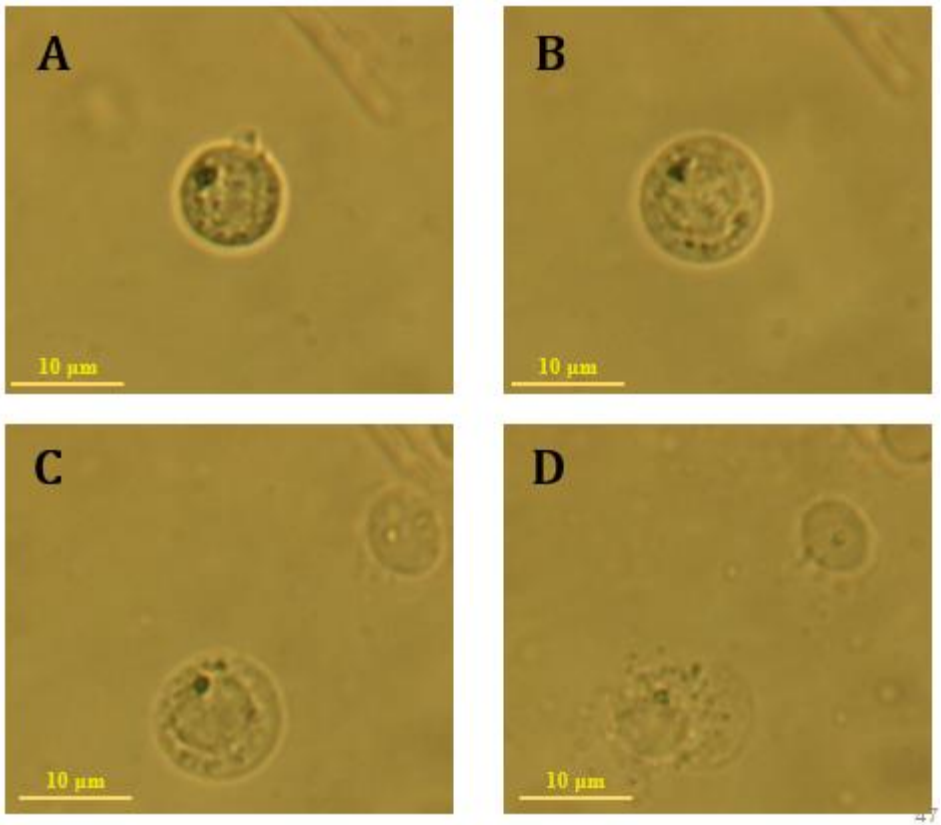


Figure 3.6 Floating MCF-7 cells in a DI water environment

A: Attached cell area before injecting DI water.

B: After 30 s. C: After 60 s. D: After 90 s.

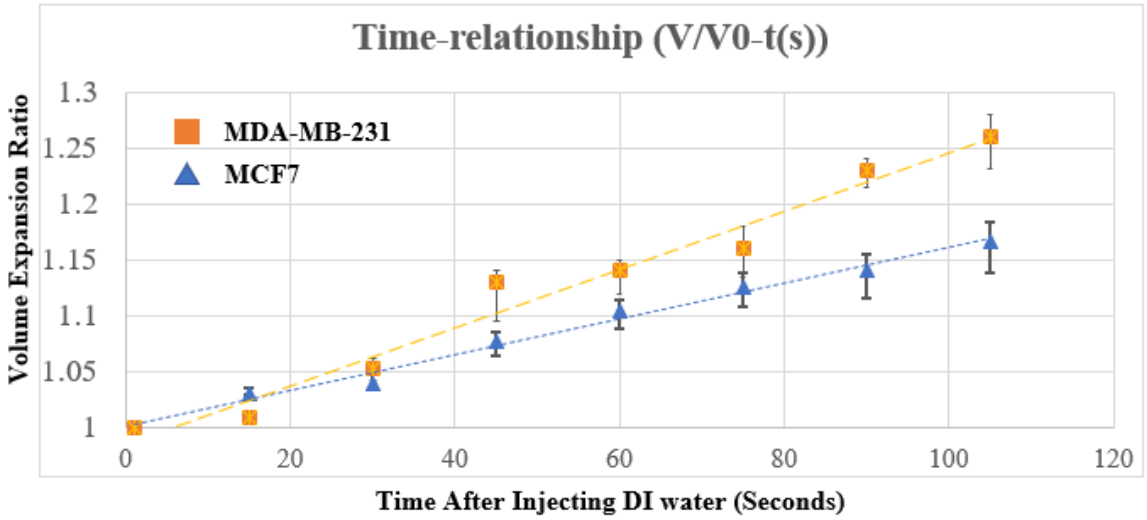


Figure 3.7 Volume change of floating MDA-MB-231 and floating MCF-7. The blue line is the floating MCF-7. The orange line is the floating MDA-MB-231.

After comparing the changing rate of MDA-MB-231 and MCF-7 in a hypotonic environment, MDA-MB-231 might have faster and more significant volume differences, which means when researching cell volume change, choosing MDA-MB-231 might observe a clearer phenomenon (Figure 3.6, Figure 3.7).

## 3.2 PDMS-free microfluidics devices under a microscope

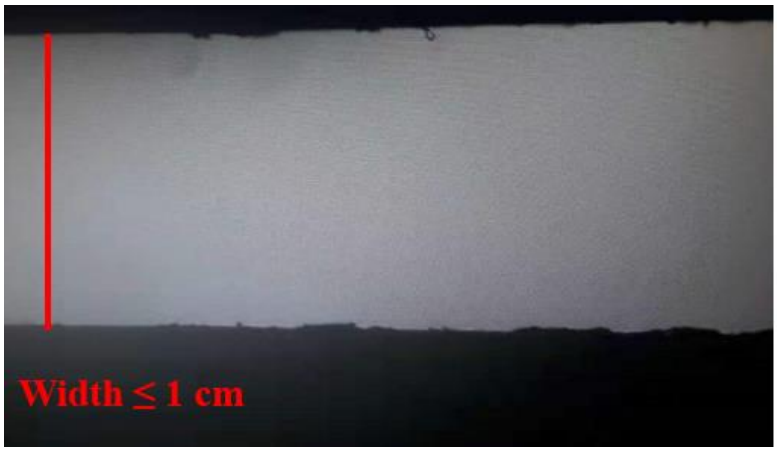


Figure 3.8 The shape of microfluidics devices under a microscope (×10)

We can successfully make a microchannel without soft lithography, and 3D bioprinting. Using our method makes it way much easier to create a microfluidic device, while the width of the microchannel is well-controlled at less than 1 cm (Figure 3.8). Moreover, by running experiments in a parallel microchannel, it is possible to control for variations in experimental conditions and to ensure that results are consistent and reproducible.

## 3.3 Cell growth in the microchannel

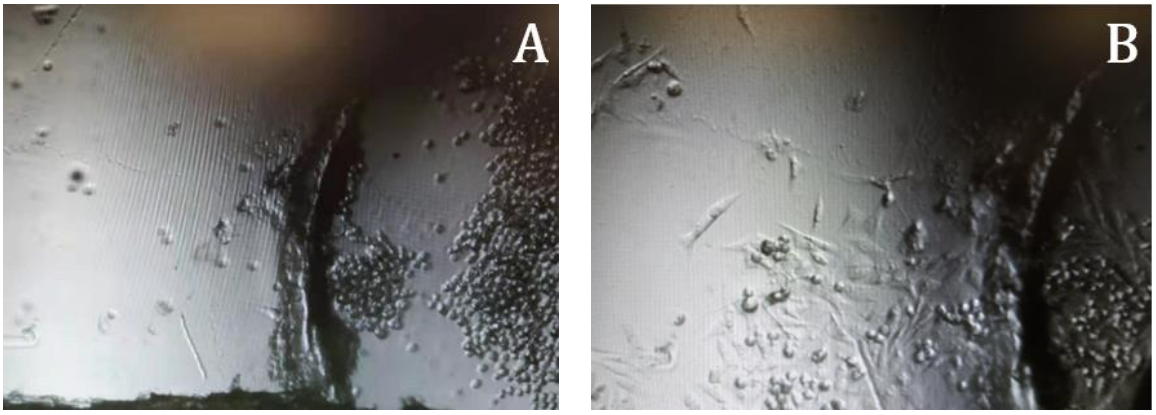


Figure 3.9 Cell performance in the microfluidics device.
A: Inject the cell in the microchannel and outlet area for 10 mins.
B: Inject the cell in the microchannel and outlet area for 24 hours.

After putting the MDA-MB-231 suspension into the microchannels (Figure 3.8), we block the microchannel to keep the environment still. To reduce the effect of cell proliferation, we only add DMEM without FBS so that the cells survive without increasing amounts. If cells grow within the microchannel during an experiment, uncontrollable factors, like interacting with the experiment's agents, chemicals, or biological molecules,

may lead to variability in experimental results. This risk can make it challenging to interpret the results and draw meaningful conclusions. Too many cells growing in the microchannel may also form a biofilm that can attach to the microchannel's surface, obstructing the channel's flow and affecting the microchannel's hydraulic resistance [60].

After culturing it for 24 hours, we could find the cells transformed from granular shape to spindle shape, which is the normal status for MDA-MB-231. And the cell could survive in the microchannel for at least three days and keep its shape (Figure 3.9). However, this method still needs further experiments to see if the cell can survive longer than seven days. Compared to a conventional culture dish or well plate, cells can behave differently in a microchannel. Culturing cells for an extended period can help assess their growth and behavior over time in this environment.

# Chapter 4

## Discussion

Coating the coverslip is an essential part of the experiment. Without coating materials on the coverslip, the cell might not attach to the surface and keep floating. In addition, since MDA-MB-231 is a type of attached cell line, they would stop growing and dead in a few days.

When culturing cell lines in a microfluidics device, keeping the microchannel full of a medium is necessary, or the cell lines will die because of lacking nutrition. Therefore, reservoirs on the inlets and outlets can also protect the cell. Covering the reservoir with caps is also crucial to reducing medium evaporation.

We start culture cells in the microfluidics device in the first few days. It is vital to keep the flux still, or the flow could be a potential problem in stopping the cell from attaching to the surface. Therefore, we block all the inlets in the first days to prevent the cell from the flow. As a result, all the MDA-MB-231 cells would attach to the surface after 7-9 hours. After that, removing the blockage and starting experiments is possible.

Osmotic pressure is an example of a biophysical cue that modulates cell function. Osmotic pressure changes have been widely associated with growth arrest and proliferation in plants and seeds and, more recently, human cancer cells. In human physiology, osmotic regulation is present in multiple phenomena. At a cellular level, osmoregulation is a crucial phenomenon that allows cells to respond to changes in concentration of the environment solution in their microenvironment through transmembrane proteins responsible for water transport. One possible mechanism linking osmotic regulation with cell behavior could be the cell and nuclear size. In the context of mesenchymal stem cell biology, it has been shown that variations in external osmotic pressure can induce changes in cell volume, intracellular molecular crowding due to water efflux, changes in cell stiffness, and ultimately impact mesenchymal stem cell differentiation in the osteogenic lineage. Sterile DI water provides a substantial osmotic pressure difference. We could observe the cell volume experience a dynamic volume change in the first few minutes. Then the volume reaches a plateau because of the cell membrane's limitation and the osmotic pressure reduction. It achieves the expected result, which shows the current coating method works well in the petri dish.

We establish a primary method for the environment of specific cells and a foundation for the future research plan to test the function of aquaporins and successfully culture MDA-MB-231 in a PDMS-free microchannel. As we showed in this paper, the microchannel could support the subsequent seeding of cancer cells.

There are several directions for future research, like switching the experiment from a static system to a dynamic system, using different liquids to stimulate the human blood vessels, and doing the following experiment with the beforementioned external stimulus Chelerythrine and Carbonic anhydrase II, or applying the advanced optical instruments like LIFPA and STED.

# Chapter 5

## Conclusion

The microfluidic chip can provide a suitable growth microenvironment for cell culture in vitro. A series of PDMS-free microfluidic chips for cell culture have been designed and manufactured, providing a stable environment for cell culture in vitro. The chip comprises PMMA and a culture chamber made of double-sided tape. The overall structure of the chip has H-type and Y-type. After the external medium enters the microchannel under the action of a driving force, the excess culture medium is stored in the reservoir. The stability of the field in the culture chamber is greatly improved. The results show that the culture chamber on the microchannel is uniform and stable, which can provide a near-body microenvironment for cell culture in vitro. Our new microfluidic device approaches have enabled the discovery of novel mechanisms of cell culture in a microchannel and led to new insights into the control of tumor growth and invasion.

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