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Design and Fabrication of Deformable Microfluidic Device for the Study of Living Cells under Mechanical Solicitations

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ABSTRACT

In living organisms, cells are often encountered with compaction, tension and shear. Most cells are intangible and balanced when a solid or seminal stratification (monolayer culture) is connected. In distortion reaction, cells also experience intense biochemical changes. Stressed cells may induce dramatic changes of the cells shape, as well as in biochemical reactions. These microsystems can meet broad applications in the biomedical research field since deformation, compression or fluid flow have been found to induce biochemical changes in cells derived from various tissues. The aim of this project is to design and to develop a microfluidic device, which allows for the culturing of adhered cells in microfluidic chambers (micro-wells) while controlling, at the micro scale, the mechanical deformation applied on the substrate on which cells are attached. In this work, a Polydimethylsiloxane (PDMS) double layered microfluidic device was designed and fabricated enabling controlled micro-sized deformation of the cell culturing micro-wells. A good agreement was found between measured deformation values and simulations.

Keywords: MDA-MB-231; Micro-sized deformation; PDMS

1. Introduction

The work involved in this research was mainly inspired by the uprising success in the new research field of the so-called Organ-on-a-chip technology [1]. The state of art in the future development of this technology is envisioned not only by a complex "Human on a chip", but also for a better understanding of tissue and cells in vivo.

Organ-on-chip can replicate key functional units of living organs in-vitro to reconstruct

integrated human organ-level physiopathology [2]. These are the micro-engineered bio-mimetic micro-devices which can be used to test efficacy and toxicity of drugs and chemicals and to create in vitro models of human diseases. Organ-on-chip represents cost-effective alternatives to conventional animal models for pharmaceutical, chemical and environmental applications. For instance, there has been an increasing interest in the development of in vitro models of human

intestinal functions, including cell culture systems that utilize trans-well filter which enable trans-epithelial barrier and transport studies and miniaturized microfluidic models that also support long-term culture [3]. Most of the existing in vitro models do not recapitulate the mechanically active micro-environment of the human organ. By ceasing peristalsis-like motions while maintaining luminal flow, lack of epithelial deformation was shown to trigger bacterial overgrowth similar to that observed in patients with ileus and inflammatory bowel disease.

The micro-system design incorporated multi-layers of closely apposed micro-fluidic channels separated by a thin porous membrane coated with ECM and lined by human epithelial cells enable analysis of epithelial barrier functions in vitro [4,5]. This device will allow for studying simultaneously cultured cells' characteristics under different experimental conditions since cells will be confined in micro wells and trapped in isolated droplets. Epithelial cells will be cultured in the micro wells so that they can face the mechanical solicitation under different environmental conditions. Two piston based united-vacuum chambers on both sides of the main cell-culturing channel with sufficient pressure allows us to produce the mechanical solicitation [6, 7].

This design of two layers stacked one top of another with a droplet-based system gave the opportunity to observe the development of epithelial cells. Two vacuum chambers on two sides enable to produce different combination of pressure on the wells and to mimic the proper mechanical solicitation on epithelial cells in different part of human body. Micro-fluidic devices can facilitate the study of mechanical regulation of human organ [8-10]. The novelty of the present work is inherent in droplet microfluidics. The existing work [1, 4] performed their

experiments with the flow of biological fluidics. However, in this paper authors used the droplet technologies and anchoring techniques.

2. Materials and Methods

Theoretical background

The Force per unit length, f , to deform the wall of Vacuum chamber originates from the pressure difference ΔP across the width w of the chamber [11].

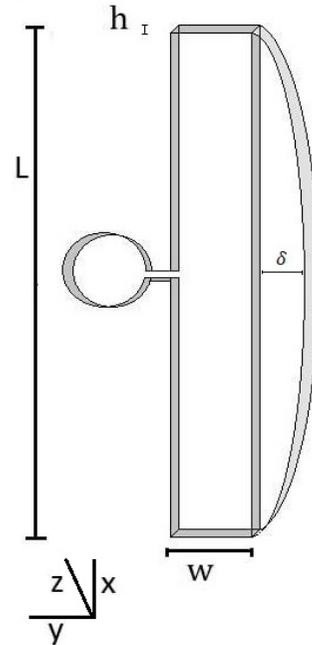


Figure 1. Deformation of vacuum chamber due to the applied force

For the height h of the vacuum chamber the deformation force per unit length, f is,

$$f = \Delta P h \quad (1)$$

It can be referred to the Euler-Bernoulli Eq. for the Deformation of the vacuum chamber.

$$EI \left(\frac{d^4 v}{dy^4} \right) = f \quad (2)$$

In Fig. 3, L is the length of the vacuum chamber, W is the width; h refers to the height and δ refers to the maximum deformation. In Eq. 2, E is Young's modulus, I is the moment of inertia and v is the deformation. For a rectangular shaped vacuum chamber the moment of inertia,

$$I = \frac{W^3 h}{12} \quad (3)$$

Droplet Microfluidics

Microfluidics is an important and relatively new tool that emerged around 1975 and which find applications in many different scientific and technological areas.

More particularly, droplet microfluidics allows for the production and manipulation of highly monodisperse microdroplets at KHz rates. They are generally made of the dispersion of an aqueous solution in a continuous non-miscible inert carrier oil phase. The study of droplets formation has been an important field among others hydrodynamics instabilities.

In this study, flow-focusing configuration is used. It consists of two immiscible liquids that are injected together in a micro channel. Therefore, one of the liquids flows coaxially into the other, the central liquid breaks into drops when the capillary forces are bigger than the viscous forces, generating at the microfluidic chip nozzle droplets with a monodisperse size. The main problem when making droplets is the coalescence between them. The use of surfactants becomes essential in order to avoid this and make droplets easily. Surfactants are molecules formed by two parts, one hydrophobic and one hydrophilic. These molecules create a layer at the interface between the two immiscible liquids that prevent coalescing of the drop with another one, even for high pressures.

The use of surfactants affects the interface properties such as the surface tension [12].

3. Microchip Design

The fabricated microfluidic device consists of two stacked PDMS micro-patterned layers: 1) Cell-dispensing droplet generator layer. 2) Layer of wells. Fig . 2 shows Cell-

dispensing droplet generator layer has a chamber where drops. These layers have two opening for the main chamber, one opening is used as inlet and another is used as outlet for cell culture medium. For mechanical solicitation, both of the layers have two vacuum chambers. Vacuum chambers are in the same length of main chambers. They are used to make the mechanical solicitation respecting the piston law.

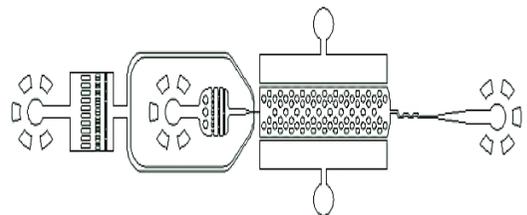


Figure 2. Top view of two layers stacked microfluidic device

The main device is comparable to 1 euro cent coin. Fig. 3 shows the main device inside the green rectangle. From the Fig. 3 a comparison can be made between the length of the device and 1 euro cent coin (16.25 mm diameter).

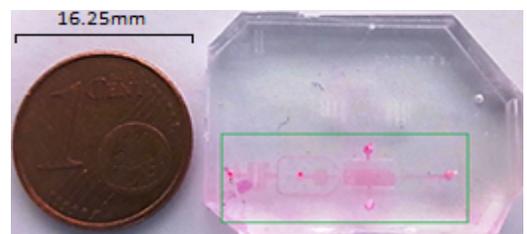


Figure 3. Comparison between the length of the device and the diameter of 1 euro cent coin.

The gradual increment of pressure force in the range of 0 to 2000 millibar was introduced. The droplet generator can create mono-disperse droplets. The nozzle size is in 100 μm and the average droplet size is in the range of 90 μm diameter. Because of the mono dispersity property and for controlled droplets making facility,

flow focusing (FF) technique was chosen. In flow focusing technique one inlet is used to flow water another inlet is used to flow oil. Droplets are made in a way like oil is cutting water. The membrane has 78 wells. These wells are distributed in a way that they follow the honeycomb structure. The honeycomb structure allows the membrane to reach minimal weight and maximum strength. This structure provides relatively high compression properties and shear properties [13]. These layers have a common main chamber where cells can be cultivated. The mechanical solicitation makes deformation in the range of cells diameter [14].

4. Fabrication

To make the mould, SU-8 (2075) photoresist was used. It is a resin which polymerizes when exposed to ultraviolet light. To give resonance to the shape of the channel, it is necessary to use a mask, which was the negative image of the design. This mask is designed AutoCAD software and then printed in a transparency sheet. High contrasts of masks are mandatory, especially for thick channels. The mould is fabricated by coating a thin layer of SU-8 on a silicon substrate which is soft baked to harden the resin, the resin stays 6 min at 65°C and then 3 min at 95°C [15]. Then the resin is exposed to UV light using the aligning machine MJB4 (in the clean room of "Institut d'Alembert", École Normale supérieure Paris-Saclay"). MJB4 will also allow the alignment of two layers. Once the design is transferred a second bake has to be done at same temperatures before the developing process. Two layers are required for this research work. For this purpose, the second layer of the same order is used on the first layer of the same level before the development process. Aligning mark according to the first and second layered

designs was added to compare the second layer design with the first layer. To align the two designs, specific alignment marks were used on both of layers. MJB4 was used to ensure the alignment of these two layers. Then the next process was to reveal the mould by a special SU-8 developer.

Moulding, bonding and channel silanization

Polydimethylsiloxane (PDMS) is a popular material used to fabricate microdevices, its properties make it highly suitable for almost any applications [16]. It is optically transparent to wavelengths bigger than 280nm which make it perfect for this study and it is convenient to use with cells and bacteria for potential applications. To make channels we mix a 10: 1 ratio of PDMS and the cross-linking agent were used. During the mix air bubbles are unavoidable. To take all the air out, the mix is introduced in a vacuum chamber until all the bubbles disappear. The mix then is poured into the mould that has been previously placed in an aluminum container. After pouring all the PDMS over the mould some bubbles will appear, so it should wait some time until the air bubbles disappear. Once all bubbles have disappeared the PDMS is cured in a convective oven for 2 hours at 75°C [2]. When the curing time is over, the PDMS is cooled at room temperature, then it is cut into the desired shape to make the inlet and outlet holes. To close the channel, it should be meticulously cleaned using 3M "magic tape" to avoid any impurity during the bonding process. The channel is covered using a carefully cleaned microscope slide for one case. For other cases, the channel is covered using a cleaned slide of PDMS. The PDMS is attached to a microscope slide for the second case. For the third case, the PDMS is not attached to a microscope slide. The two pieces are adhered using plasma

bonding. The surface of the two pieces is activated using a ‘Harrick’ Plasma Cleaner chamber. This will change the surface creating Si–OH groups on it which react with each other when the two surfaces are put on contact, creating a covalent link. Once the channels have been exposed to the plasma cleaner, the surface is no longer hydrophobic, so it is necessary to make some treatment to recover the hydrophobic proprieties of the PDMS and avoid that the aqueous phase sticks to the walls. To make this, the channels were coated with silane [17]. (This process was done in the laboratory of “Le Laboratoire de photonique quantique et moléculaire (UMR 8537), École Normale supérieure Paris-Saclay).

5. Simulation

The deflection of well can be found by simulating the design in multi- physics simulation software. Design from CAD software was called in multi- physics simulation software. This design was transferred into three-dimensional versions.

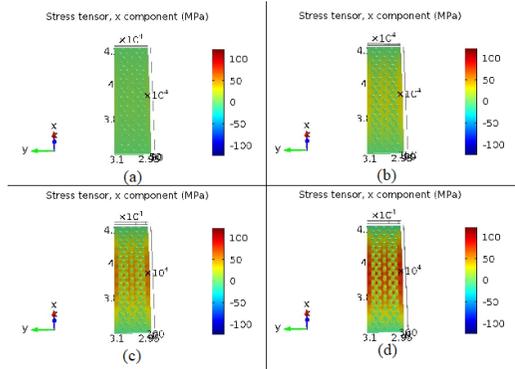


Fig. 4. Simulated deformation of wells under different pressure force applied. (a) 0 mbar which was taken as reference, (b) under 800 mbar, (c) under 1600 mbar,(d) simulated deformation of wells under 2000 mbar.

With proper Multi-physics module, applied pressure force form zero millibar to 2000 millibar with an increment of 200 millibar was considered on two sides of the

membrane. Figure 4 shows simulated deformation of wells under 0millibar, 800 millibar, 1600 millibar and 2000 millibar.

Figure 5 shows the graph of simulated deformation of the wells along x-axis under different pressure force applied. In case of simulation, the deformation of wells follows the Eq. (5) and for the increment of 200millibar the deformation increases in average of 10um.

In Simulation, the boundary condition on Zero deflection and Zero moment, $v(0) = 0$ and $v(L) = 0$.

$$y = 0, \left(\frac{d^2v}{dy^2}\right) = 0;$$

$$y = L, \left(\frac{d^2v}{dy^2}\right) = 0;$$

$$y = \frac{L}{2}, \left(\frac{d^2v}{dy^2}\right) = 0.$$

Integrating Eq. (2) with respect to y using boundary condition,

$$v(y) = \frac{L^3y - 2Ly^3 + y^4}{24EI} \quad (4)$$

Differentiating Eq. (4) and using boundary Eq. , we can also find the maximum deformation, δ

$$\delta = \frac{5PL^5}{384EI} \quad (5)$$

Considering, $K = \frac{5L^5}{384EI}$, in Eq. (5), $\delta = KP$. Here, maximum deformation is directly proportional to the applied pressure force.

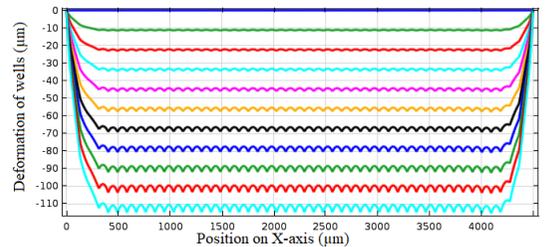


Fig.5. Deformation of wells along X-axis

6. Experimental Deformation

Fig. 6 shows the schematic diagram of the experimental setup. In the experiment a

program was used which allow to increment the pressure after a specific amount of time.

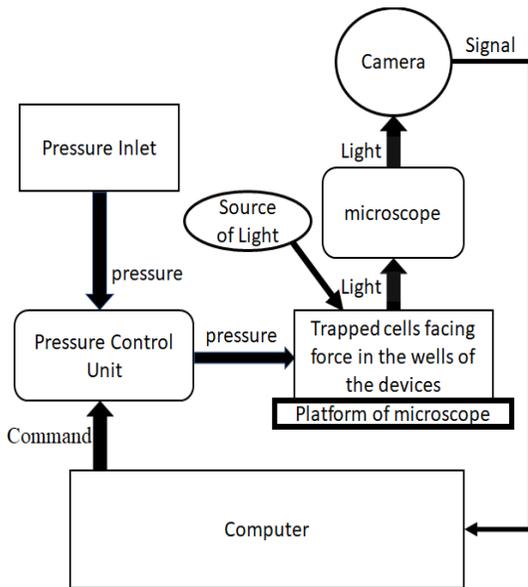


Figure 6. schematic diagram of experimental setup

In order to find the best experimental conditions, deformations with three different approaches were investigated: (1) PDMS device sticking to the glass, (2) PDMS device sticking to a thin layer of PDMS which is also sticking to the glass, (3) PDMS device sticking to a thin layer of PDMS which is not sticking to the glass were applied.

In case of approach (3), comparatively better deformations were achieved. Pressure force was applied in the range of 0 to 1800 millibar with the gradual increment to 200 millibar. Deformation can be seen for 0 millibar and 2000 millibar in Fig.7. During the experiment, below 800 millibar no deflection was found. Table 1 shows that the deformations are in μm range which is equivalent to the diameter of the cell. Impulse and gradual increment of pressure force were applied for the minimum amount of time (sec). In both cases, threshold pressure forces were found. The threshold pressure force for impulse pressure force

applied, P_T is much lower than threshold pressure force for the gradual increment pressure force applied, P_G .

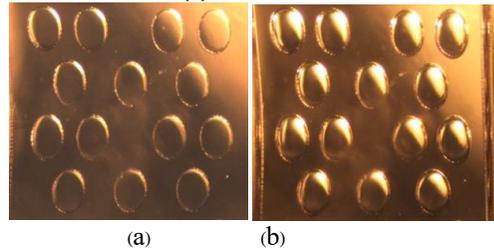


Figure 7. Deformation of wells during experiment under pressure force applied. (a) 0 millibar which was taken as reference (b) 2000 millibar.

Table 01: Deformation of well

Pressure Force applied(millibar)	0	800	1600	2000
Deformation (μm)	0	3.721	6.082	10.162

6. Cell Culture

MDA-MB-231 cells were used in this experiment. The device was prepared by exposure of UV light about 5min to activate the surface of the membrane. Then 4 percent of collagen (ECM) was applied for about 60 min. Phosphate Buffered Saline (PBS) or cell culture medium can be used to clean the device. During the cell culture, cells are bonded to the surface with antigen (Protein) and when Trypsin was applied, it breaks the bond. After cells started to flow, cells were placed for 4min in 37°C [18]. Then the cells were transferred in plastic Lab tube container. Centrifugal force of about 100r.p.m. was applied to the cells for 5 min. Then cells were found at the bottom of the plastic Lab tube [19-22]. Cells can be introduced with cell culture medium in the droplets with the help of Drop-maker. Cells with cell culture medium can replace the disperse phase [23]. Oil phase will cut the cell culture medium and thus make droplets with cells. Poisson's Distribution Law can

provide the number of cells in a droplet. Fig. 8 shows that the droplets were anchored to place the cells on the surface of the wells of the membrane.

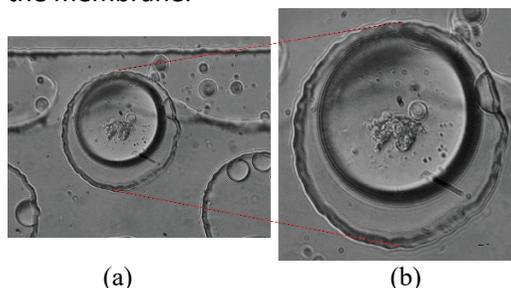


Figure 8. (a) Anchored cells in well, (b) zoomed view of droplet with cells in well.

Figure 8 shows, anchored droplets with cells. Cells are trapped in the wells to be submitted to mechanical solicitations. The gradient of surface energy and hydrodynamic drag anchors the droplet in the wells [24, 25]. Fluorinated oil has advantages of absorbing O_2 and CO_2 . This will help to flow O_2 and CO_2 in wells that will enable cells to live and grow in this environment.

7. Conclusion

This paper presents the development of a microfluidic device that can be helpful for a long range of biological study even including high throughput experiments. It describes the methodology for the construction of the presented device clearly. However, in real experiment, deformation was multiple times smaller. According to Eq. (5), during small pressure change, there should have been bigger deflection. However, mathematically deformation should be proportional to the applied pressure force. In experimental deformation, it is also found that after crossing the threshold pressure force the deformation is directly proportional to applied pressure. In the simulation, it is found that deformation also follows a direct proportionality with applied pressure. In the results, due to experimental

limitation, the data is subjected to the error of $(+/-)1\mu m$ [26]. The application of droplet microfluidics and continuous phase flow with mechanically deformable 3D microfluidic chip allows making deformation of approximately $10\mu m$. The use of fluorinated oil facilitates the growth of cells and a good living condition for them. The challenges in microfluidic design with mechanical solicitation are the formation and adjustment of micro-droplets, trapping or anchoring the drops with cells and control the number of cells per drops. Using PDMS gives facilities such as optical stiffness, high gas permeability and most importantly biocompatibility [27]. Moreover, microfluidic production of droplets can be scaled up to a high throughput platform offering great potential for numerous applications in life and material science. Author's future work will be devoted to testing the device with cells for a long period of time for various chemical solutions. This work is a beginning for building a device which can be used to prescribe the specific dose of medicine for an individual patient.

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