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Gelatin Methacryloyl Hydrogel Compartments for Microfluidic Devices

THESIS

submitted in partial satisfaction of the requirements
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MASTER OF SCIENCE

in Mechanical and Aerospace Engineering

by

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Professor Yun Wang

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ABSTRACT OF THE THESIS

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Professor Abraham Lee, Chair

Microfluidic devices scale down to the size of a single cell, allowing studies in individual cells. The thesis focuses on developing a new method to create individual compartments with gelatin methacryloyl hydrogel for cell trapping and cell pairing microfluidic devices efficiently. The individual gelatin methacryloyl compartments at the cell traps and cell pairing sites allow long term cell culturing for further studies and observation of the target cell or cell pairs.

The compartments were created by photopatterning gelatin methacryloyl and photoinitiator- Irgacure 2959 mixture. The mixture crosslinked when exposed to the ultraviolet light. The light source for this project was the inverted microscope- Olympus IX51. Instead of creating a compartment one site at a time according to previous work, the new method allowed the process to be done in one or two exposures within 30 seconds and created 50 to 100 compartments. After experimenting on the testing microfluidic device, the procedure was finalized. The method could not create the same patterns on the devices with microstructures. A possible cause of the unsuccessful photopatterning is the refraction of light through polydimethylsiloxane microstructures. Future improvements of

the method are expected in two aspects. One is the refraction of light and the other is the material of microstructures. A possible way is to lower the refractive index either by changing the property of polydimethylsiloxane or change to other materials. Another possible method is to use gelatin methacryloyl as the material of microstructures.

CHAPTER 1: Introduction and Background

1.1 Introduction

Microfluidic devices allow the research to be done on a single-cell scale since the dimensions of the microstructures can reach the size of some types of cells. Other advantages of microfluidic devices include lower power consumption, less amount of sample required, faster reaction, being portable and disposable. They are also easier to operate and have fewer restrictions on the environment. There are some techniques in microfluidics to capture and manipulate single cells and enable analyses of single cells instead of analyzing the bulk. The cells are not identical, but conventional analysis of the bulk will only show the average response of all the cells and not specify the differences between each cell.

This thesis developed from previous work of two cell manipulation techniques on microfluidic devices- cell trapping and cell pairing [1]. They both used passive mechanical methods to make the cells to flow into the microwells without other external forces. This method would decrease the possible damages to the cells. In order to culture and observe the cell for a long period of time, GelMA was used to fabricate individual compartments for each cell or cell pairs. The use of gelatin methacryloyl hydrogels (GelMA) on microfluidic devices enable more function on the device without damaging the cells. GelMA and photoinitiator- Irgacure 2959 mixture can be crosslinked when exposed to the ultraviolet (UV) light. It was chosen as the material for this thesis because of its good biocompatibility and cells encapsulated in GelMA showed high viability [2]. The previous method was to

create the compartments one at a time [1] which made it time consuming and labor intensive to create large single cell trapping arrays. There are 50 to 100 compartments on one device depending on the design, so it would require 50 to 100 times of exposure which takes 15 to 30 seconds each.

The experiments were done to test out the ratio of mask pattern dimension to GelMA pattern dimension, the proper objective lens of the microscope to use, the amount of exposure required, the acceptable length of time for each exposure, the material of photomask for photopatterning GelMA, and the photomask design for cell trapping and cell pairing microfluidic devices.

1.2 Microfluidics

Miniaturized devices developed from electronic devices with integrated circuits to other devices with variations in mechanical and electrical designs. The advantages of miniaturization include lower energy consumption, fewer materials required, lower cost, being disposable and portable. In some cases, the physical phenomena change due to scaling law is favorable and the sensitivity of the device is higher [3].

Microfluidics is the study of fluidics in microscale. Similar to other miniaturized devices, microfluidic devices also benefit from their size. There are scaling of physical quantities that are specific for microfluidics. The scaling is important when determining the methods to introduce, move, and mix the reagents and samples [4]. In microscale, Reynold's number is low in the laminar flow range. There is no convection of fluid and the only mixing mechanism is diffusion which makes it more predictable. Different from macroscale, quantities such as surface tension, interfacial tension, and capillary forces

dominate over gravity in microscale allowing the formation of bubbles, droplet microfluidic and the mechanism to move fluid passively [5].

Microfluidics can be applied in many fields such as optical systems, cell biology, chemical synthesis, and bioanalysis [4]. The microfluidics devices used in this thesis are in the field of cell biology for manipulating single cells. The nominal dimension of the microstructures on the fabricated devices is around 15um which fit the targeted types of cells, so the separation and pairing of each individual cell can be achieved for further studies.

There are some materials that are used to fabricate microfluidic devices including polydimethylsiloxane (PDMS), thermoplastic, paper, wax, and cloth [5]. In this thesis, PDMS is used to fabricate microfluidic devices. The use of PDMS as the material for the structure provides a suitable environment for growing and observing cells due to the characteristics of PDMS including transparent, low toxicity, and high permeability to oxygen and carbon dioxide [4]. PDMS microfluidic devices are fabricated by the process of soft lithography. There are two types of PDMS microfluidic devices for the single-cell study being used in this thesis- cell trapping array and cell pairing microfluidic devices.

1.3 Single-Cell Manipulation and Analysis

With our microfabrication technology, microfluidic devices can achieve the trap single cells nominally with diameters of 15 μm. Traditional analysis is based on a population of cells and ignoring the difference between each cell. Therefore, the results are only based on the average response of the bulk [6]. In order to have accurate results

reflecting the characteristics of each cell and to observe the reaction between cell pairs, the development of robust single-cell analysis devices is crucial.

There are different single-cell manipulations [6] including single-cell encapsulation [7], sorting [8], trapping [1], isolation [9], rotation [10], pairing [1], patterning [11], stretching [12], transportation [13], lysis [14], and stimulation [15]. The thesis will focus on single cell trapping, pairing, and encapsulation at the trapping and pairing sites. There are methods of manipulations such as hydrodynamic, electrical, optical, acoustic, magnetic, and micro-robotic [6]. The mechanical method is one category of the hydrodynamic methods. There are active mechanical systems using microvalves and passive mechanical systems using microstructures [6]. The passive mechanical method provides minimum interference and damage to the cells when there are only microstructures that allow cells to flow in passively and no additional external forces exert on the cells. The devices used in this thesis contain microstructures- microwells to capture one cell in each microwell passively. Single-cell analyses such as cellular analysis, genetic analysis, protein analysis, and biophysical property analysis [6] can be done on the integrated microfluidic devices.

Cell-cell interaction study is another field that benefits from single-cell manipulation techniques and microfluidics. Cells can be paired with the technique by separating a single cell from the bulk and pairing with another cell. The resulting response will be only based on the cell pair like other single-cell analysis instead of the whole population. The research [1] focuses on the interaction between the dendritic cell and the cancer cell. By pairing the two types of cells, the response of the immune system to the cancer cell can be observed.

As in this thesis, the microstructures- microwells of the size of a single cell are designed to be placed in each channel and provide arrays of traps for the cells to be

captured. As the cells flow through the channel, they will be hydrodynamically trapped in each site. The following are two types of devices for single cell studies.

1.4 Microfluidics Cell Trapping Device and Cell Pairing Device

The microfluidic cell trapping device in this thesis is based on a recent study [1]. It contains 100 microwells where 20 microwells are in a row and there are 5 rows of serpentine channels [1]. The size of each square microwell will be designed to fit the diameter of the target cell. The design of the cell trapping device is shown in Figure 1.1. The sample will be injected at the inlet on the top left, the cells will be trapped as they flow through the channel.

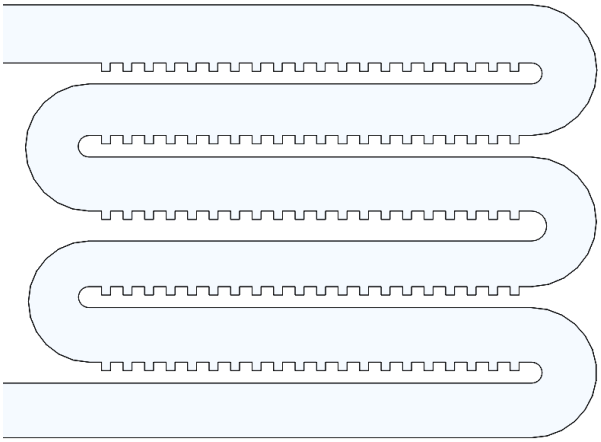


Figure 1.1 Schematic of Cell Trapping Device.

In addition to the cell trapping device, a microfluidic cell pairing device was also used in this thesis. The design was from the study [1]. It was the modification of cell trapping design. Two microwells were combined in the opposite direction with a small opening in between. The simplified schematic is shown in Figure 1.2. The length of the cell

pairs is 30 μm and the width of microwells is 15 μm. There are 10 pairing sites in each row and 5 rows in a device. The working principle is slightly different from the cell trapping device. First, one type of cell is injected into the device through the forward flow, and trapped in the first microwell of the pairing site. Then, the other type of the cell is added through the reverse flow and trapped in the other microwell at the pairing site.

Figure 1.2 Schematic of Cell Pairing Device.

In order to achieve long-term culturing of the cells trapped in the microwells, Li [1] suggested using gelatin methacryloyl hydrogel (GelMA) to create individual compartments for each cell or cell pairs which allowed the supply of media [1]. The current method is creating the compartments one at a time by exposing each microwell to UV light to crosslink GelMA and Irgacure 2959 mixture which is inefficient since there are large amounts of microwells on each device. The improvement suggested by Li [1] is to design a mask that could create the whole array of GelMA compartments at once by the inverted microscope. The objective of this thesis is to develop from this idea and design photomasks and methods to create GelMA compartments efficiently.

1.5 GelMA Hydrogel

Gelatin methacryloyl hydrogels (GelMA) is chosen as the material for individual compartments for cell culturing in the microfluidic devices because of its properties of good biocompatibility, biodegradable, non-cytotoxic, non-immunogenic, and high cell viability [2].

Microfabrication techniques of GelMA hydrogel include photopatterning, micromolding, self-assembling, bioprinting, and textile techniques [2]. Photopatterning techniques could be done with or without the photomasks. GelMA can be crosslinked when photoinitiator is added and the exposure to ultraviolet (UV) light. A simple method to create GelMA structures is by placing the photomask in the path from UV light to the device, but the structures created are limited to simple planar patterns. There are also techniques such as stereolithography and two-photon polymerization that can create complex GelMA structures layer-by-layer without photomasks [2], [16],[17]. In this thesis, GelMA is mixed with the photoinitiator-Irgacure 2959 and the UV light source is from an inverted microscope- Olympus IX51 with the photomask placed in the filter slider of the microscope.

1.6 Objectives

The objective of the thesis is to develop a faster and simpler method to create GelMA hydrogel compartments matching the design of microfluidic devices by photopatterning GelMA with a microscope and a photomask. Previous methods from [1] created the GelMA individual compartments for cells and cell pairs one microwell at a time by exposing the GelMA around that microwell to the UV light from a microscope. The area of exposure was limited

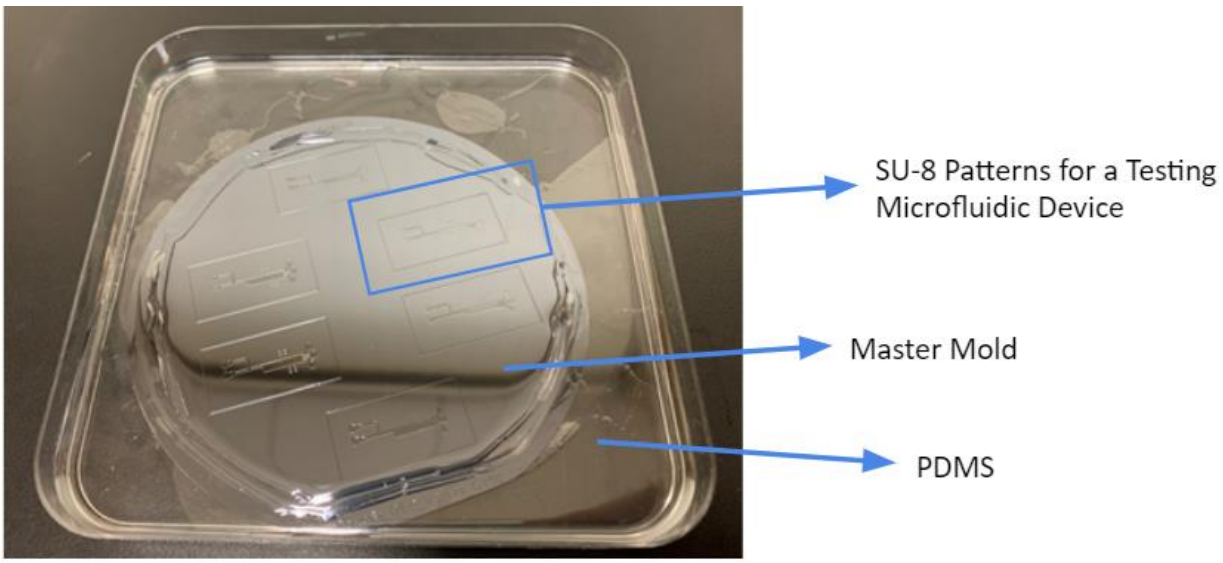
by the field iris diaphragm of the microscope. According to the suggested improvements from previous work [1], all GelMA compartments for the whole device were expected to be created at once using a photomask to block the light and allowing a certain pattern of the light beam to pass through. Based on the knowledge, the following experimental setups and materials were used in the experiments which determine the photomask design and the new fabrication method of GelMA compartments on the cell trapping and cell pairing device.

CHAPTER 2: Experimental Setups and Materials

2.1 Fabrication of Microfluidic Devices

Microfluidic devices were designed with computer-aided design (CAD) software. The CAD files were sent to the vendor to fabricate photomask for photolithography to produce master molds for soft lithography. The master molds for soft lithography were fabricated by 2-layer photolithography on a silicon wafer with patterned chrome masks. The photoresist, SU-8, was used in the process. Two of the master molds-testing mold and cell pairing mold, used in this thesis are shown in Figure 2.1. Microfluidic devices were fabricated through the process of soft lithography using polydimethylsiloxane (PDMS) as the material. PDMS and curing agent mixture were poured onto the master molds in the container and then were cut out after solidified.

(A)



(B)

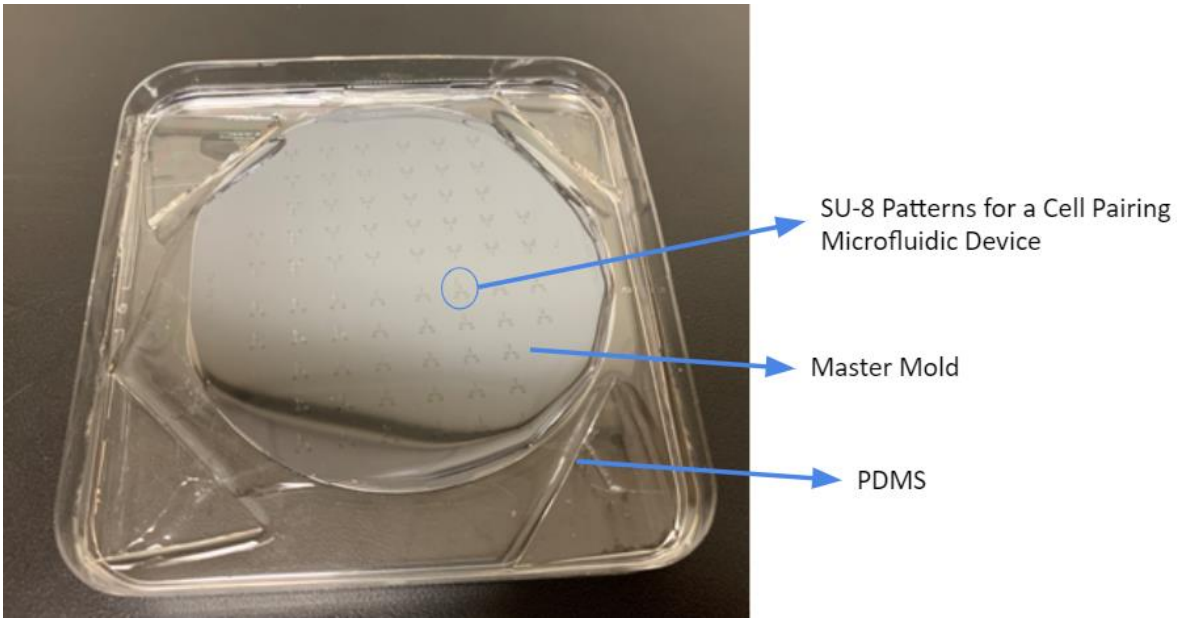


Figure 2. 1 Master molds for the testing microfluidic device and cell pairing microfluidic device.

(A) Master mold for the testing microfluidic device made by photolithography. The mold was in a container where PDMS was poured into. (B) Master mold for the cell pairing microfluidic device.

PDMS mixed with a curing agent at the ratio of 10:1. The mixture was completely mixed and put into the vacuum desiccator, shown in Figure 2.2, for an hour to eliminate bubbles. PDMS mixture without bubbles was poured onto the master mold and baked at 120 ℃ for 5 minutes. It was left on a flat surface at room temperature overnight.

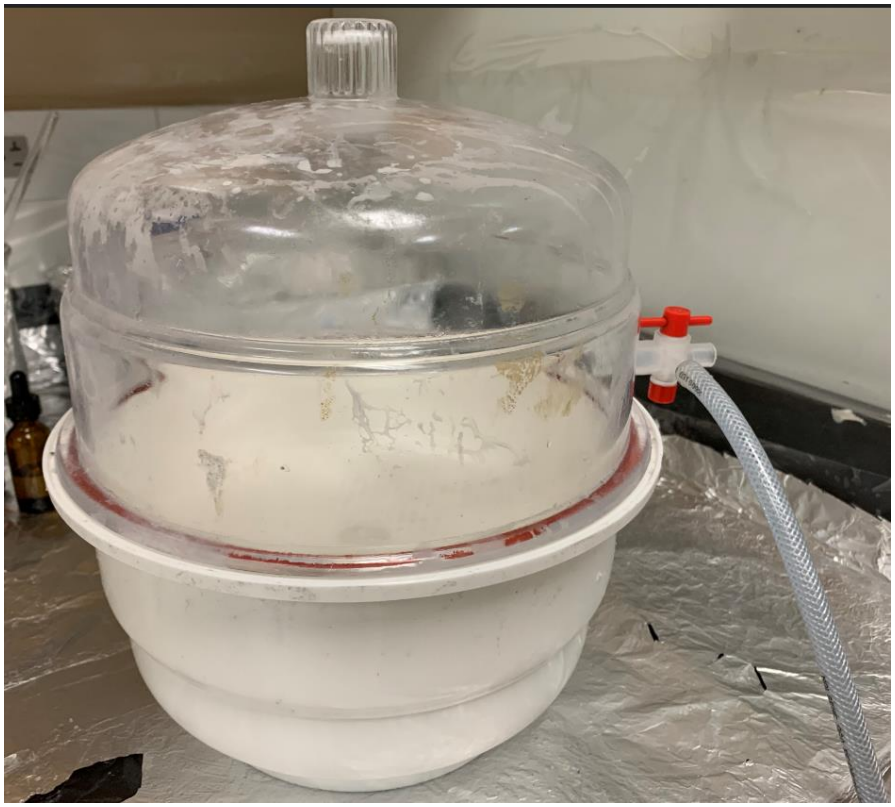


Figure 2. 2 Vacuum desiccator.

After PDMS and curing agent became solidified, it was cut out of the master mold and the inlet and outlet of the channels were punched. The PDMS part was sealed to a clean glass slide by the process of plasma bonding. Plasma bonding started with cleaning the glass slide and baking the glass slide for 2 minutes at 120 ℃. The PDMS part was also cleaned after punching the inlet and outlet with tape and nitrogen gun. Both the glass slide

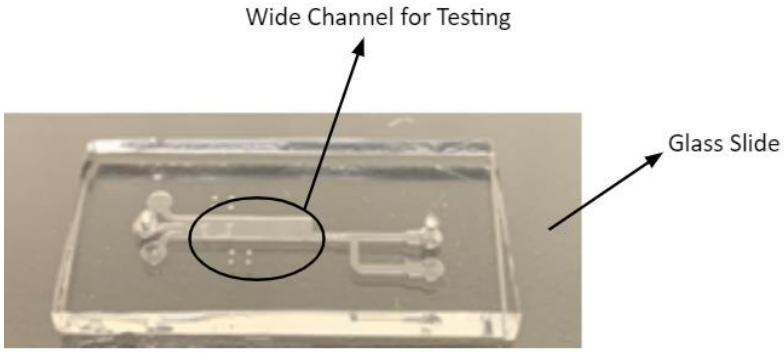
and the PDMS part were placed in the plasma chamber, shown in Figure 2.3, with the bonding sides faced up and the vacuum pump was turned on. Plasma was also turned on for 2 minutes at a power of 30 W after the chamber reached around 150 mTorr and turned back to around 250 mTorr. Finally, the glass slide and PDMS was taken out of the chamber, bonded, and the whole device was baked for 3 minutes at 120 ℃.

Figure 2. 3 Plasma chamber.

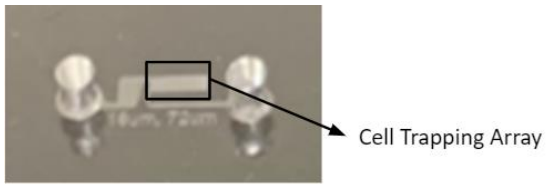
Three types of microfluidic devices were used in this thesis. One of the devices is the testing microfluidic device which contains no microstructures in the wide channel. It was used in the first part of the experiments to test out the basic parameters of the new method and the design of photomask patterns. The other two are cell trapping and cell pairing

devices. These were the actual devices that would trap cells. Three microfluidic devices are shown in Figure 2.4.

(A)



(B)



(C)

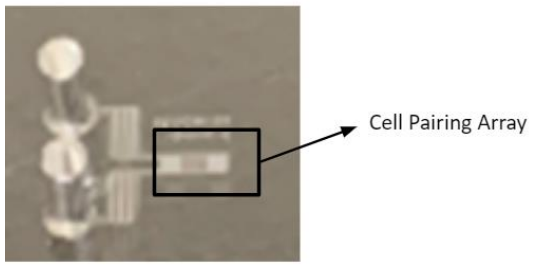


Figure 2. 4 Microfluidic devices.

(A) The testing microfluidic device. (B) Cell trapping microfluidic device. (C) Cell pairing microfluidic device.

2.2 GelMA Mixture Preparation

The mixture of GelMA hydrogel and photoinitiator-Irgacure 2959 was prepared with the following solution. GelMA and phosphate-buffered saline (PBS) were mixed to produce the 10% w/v GelMA solution. The 50% w/v photoinitiator solution was prepared by mixing Irgacure 2959 and Dimethyl sulfoxide (DMSO). The mixture for the experiment contains the 10% w/v GelMA solution, PBS and 50% w/v Irgacure 2959 solution of the ratio of 50:49:1.

2.3 Materials of Photomask for Photopatterning GelMA

The photomasks used in this thesis included two materials- the film photomasks and emulsion glass photomasks. Both of the masks contain clear patterns where light is able to go through and crosslink GelMA, and the dark background to block the light. Therefore, the clear patterns design on the photomask should match the desired patterns of GelMA. The film photomasks were used at first because they cost less and they are faster to fabricate and post-process. The film photomasks were fabricated and processed by CAD/Art Services, Inc according to the designed CAD file and the cutting size of the photomask given. After a few experiments, the lamp of the microscope reached the lifetime limit and was replaced with a new lamp. The new lamp exposed higher energy and melted the film photomask immediately. Emulsion glass photomasks were used afterward. The emulsion glass photomask was fabricated by Front Range Photomask. The CAD file and the photomask laser cutting size were sent to the vendor to produce a 32 mm glass photomask with specific patterns. Four emulsion glass photomasks are shown in Figure 2.5.

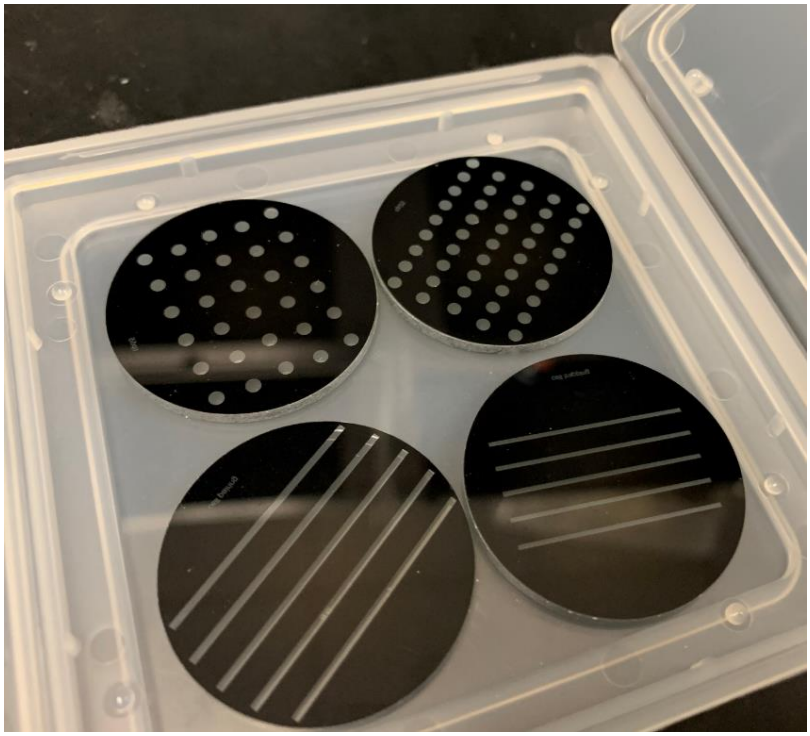


Figure 2. 5 Emulsion glass photomasks with four different designs.

2.4 Microscope Setup for Image Acquisition

The microscope used in the experiment was the Olympus inverted microscope-IX51. Different from the previous method to crosslink each pairing site one at a time when the field iris diaphragm was closed to limit the area of light, the field iris diaphragm was fully open for the new technique and the light will only be blocked by the dark background and pass through the clear patterns of the photomasks. The photomask was placed in the filter slider of the microscope shown in Figure 2.6.

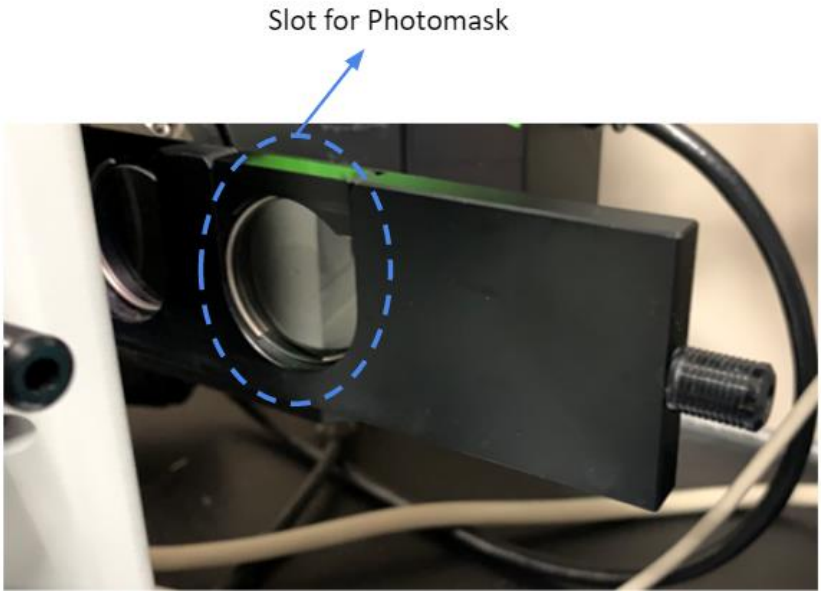


Figure 2. 6 Filter slider of the microscope.

In order to measure the size of microstructures of the microfluidic devices and to measure the crosslinked area of GelMA, the calibration slide was used to determine how many micrometers were in one pixel of the image taken by the microscope. Figure 2.7 was taken when using the 10x objective lens and 0.5x adapter.

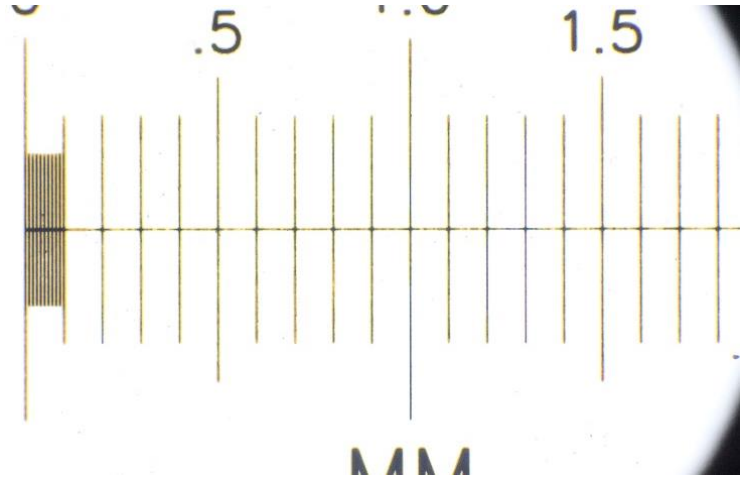


Figure 2. 7 Calibration slide image at 10x objective lens and 0.5x adapter.

After the image was taken, it was measured by ImageJ for the number of pixels in each grid and calculated the number of micrometers per pixel. The process was repeated for the 20x and 40x objective lens with the same 0.5x adapter. The objective lens of 10x, 20x, and 40x are the main ones used for this project. Table 2.1 shows the measurement and calculation results of the number of micrometers per pixel for three objective lenses.

Table 2.1 Measurement of microscope image.

Objective Lens	μm /pixel
10x	0.3397
20x	0.1645
40x	0.08417

Chapter 3: Results and Discussion

3.1 Photopatterning Designs for GelMA

The experiments were done to test out the details and parameters of the new method. They were done on a testing microfluidic device with a blank and wide channel first. The testing microfluidic device contained no microstructure in the channel. After finalizing the procedure and correcting the dimensions of the photomask, the experiments were started on the cell trapping and cell pairing devices.

At the beginning of the experiments, film photomasks were used to pattern crosslinked GelMA. It was placed in the filter slider of the microscope to selectively block the light pass through it. They worked well because the light source of the microscope had less energy when it was close to the end of the lifetime limit- 200 hours. It couldn't withstand high temperatures when the energy of the light source became higher after the new lamp was installed. The film mask melted and deformed immediately when exposed to the light. Figure 3.1 shows the deformed film photomasks and the resulting crosslinked GelMA patterns.

(A)

(B)

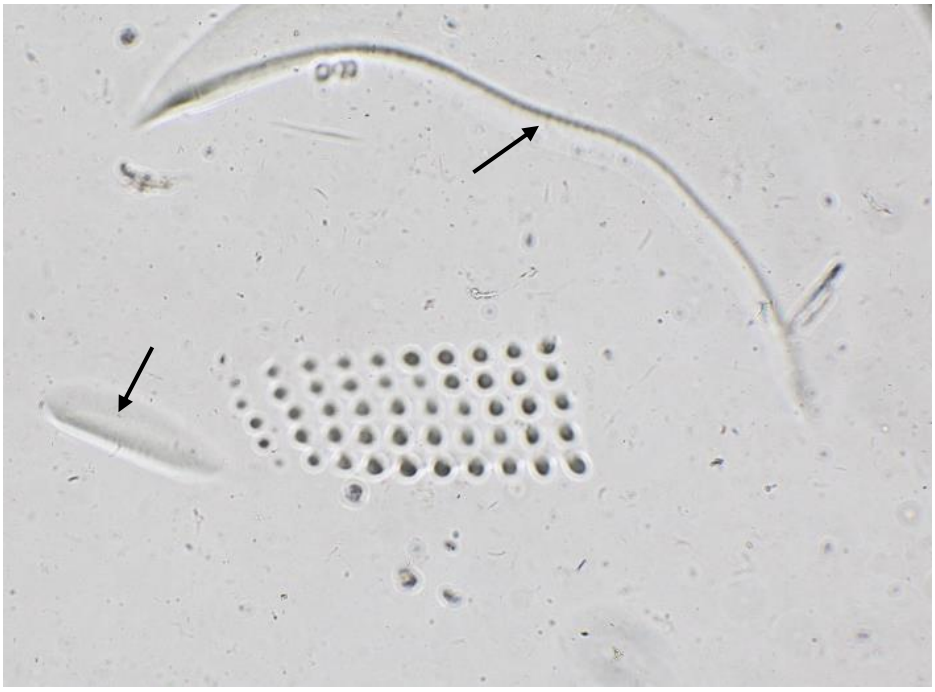


Figure 3.1 Deformed film photomasks and the GelMA crosslinked patterns from the deformed photomask.

(A)The film photomasks deformed immediately when exposed to the new microscope lamp. (B) The GelMA crosslinking process was not successful due to the deformed film photomask. The arrays of dots were tilted and the right upper part and the left part were crosslinked because the deformed photomask was bent and not blocking the light on the edge.

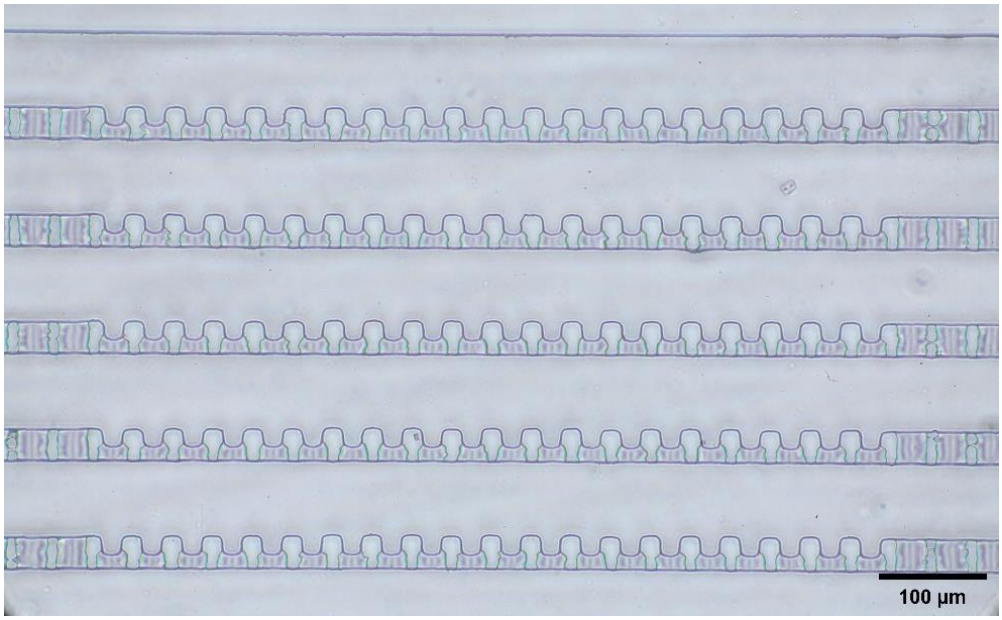
There were a few attempts trying to keep the film photomask flat and not deformed due to the heat included making the exposure time as short as possible and putting it on a flat surface with weights on top of it after used, adding a quartz disc of 1.25-inch diameter on one side of the film photomask when exposing to the UV light, and adding the quartz discs on both side of the film photomask to stabilized it while exposing to the UV light. However, the film photomask still deformed to a degree that greatly influenced the patterning of GelMA. Therefore, other materials for photomask were being considered.

There were two choices, chrome photomask or emulsion glass photomask. Since the minimum feature of the photomask was larger than 500 μm, it was unnecessary to use a chrome photomask. The emulsion glass photomask was also less expensive, so it became the new material for photomask used in this thesis. The emulsion glass photomasks were fabricated by Front Range Photomask and laser cut to circular glass masks of 32 mm diameter to fit in the filter slider of the microscope. The advantages of emulsion glass photomask were that it will not be deformed by the heat from the light source and they were easier to be placed upright in the filter slider since they were thicker than the film photomasks. The disadvantages were that the emulsion glass photomasks cost much more than the film photomasks and they took more time to fabricate and post-process.

First, the dimension of the photomask pattern and the corresponding objective lens used were determined by the experiments with different dimensions of mask pattern-circles of diameter from 0.5 mm to 4 mm, and three different objective lens-10x, 20x, 40x with the same 0.5x adapter. As shown in Figure 3.2, the whole device was in the range at 20x objective lens, but it only showed 3 rows and around 12 traps at 40x for the 5 rows of 20 traps cell trapping microfluidic device. It was the same for the cell pairing microfluidic

device, there were only 4 rows and 8 pairing sites for the total of 5 rows and 10 pairing sites at 40x and the whole device was shown at 20x.

(A)



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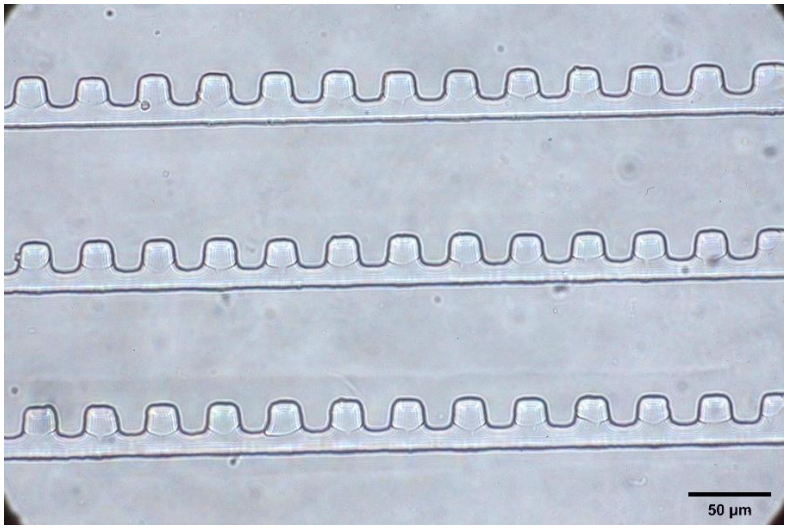
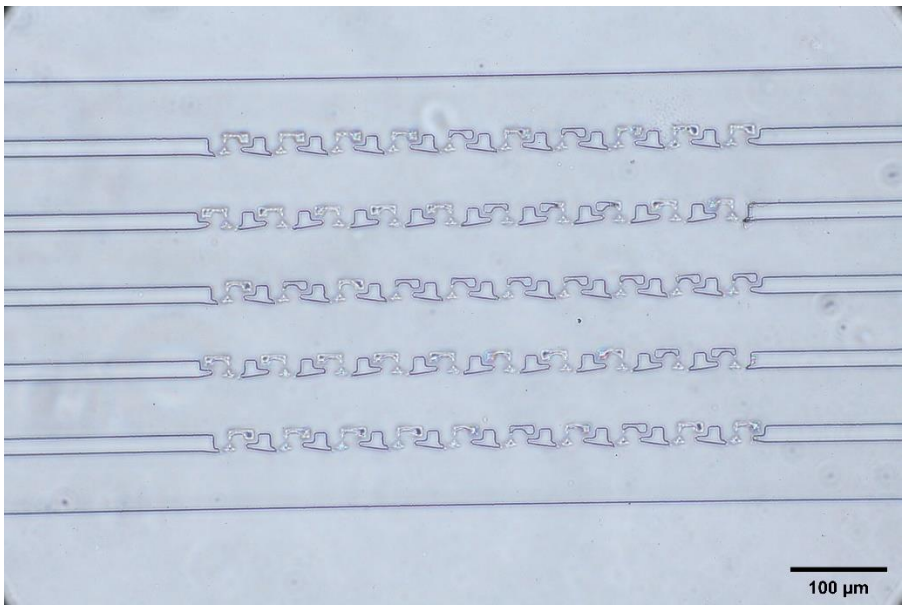


Figure 3.2 Cell trapping and cell pairing microfluidic device at 20x and 40x objective lens and 0.5x adapter.

(A) Cell trapping microfluidic device at 20x, showing all the traps. (B) Cell trapping microfluidic device at 40x, showing part of the traps.

(C)



(D)

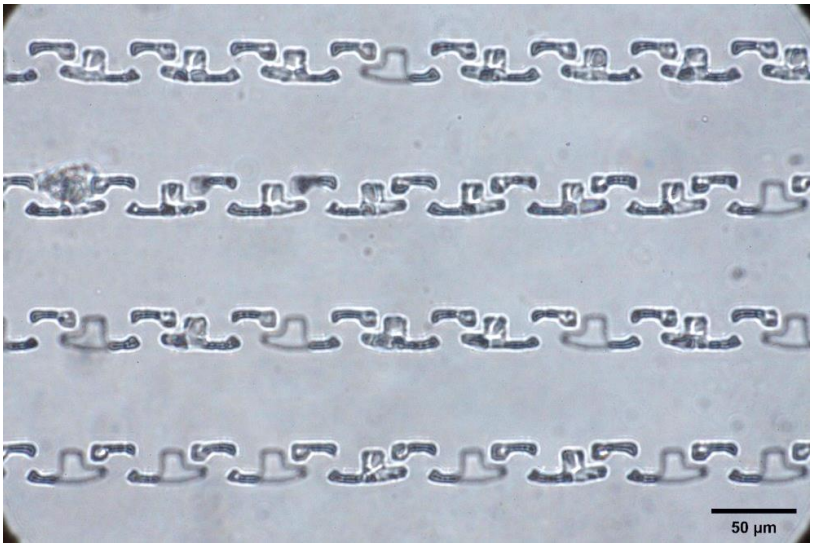


Figure 3.2 Cell trapping and cell pairing microfluidic device at 20x and 40x objective lens and 0.5x adapter.

(C) Cell pairing microfluidic device at 20x. (D) Cell pairing microfluidic device at 40x, showing part of the pairing sites.

First, the goal was to use the 20x objective lens and produce GelMA compartments for the whole device at once. However, the resolution of crosslinked GelMA patterns was not high enough at 20x (Figure 3.3). The second part of the experiments was done using the 40x objective lens.

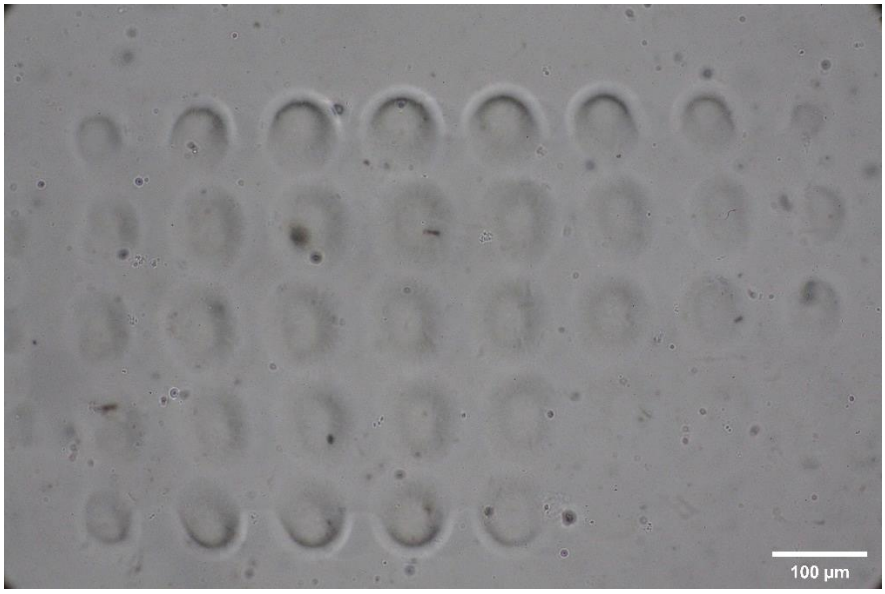


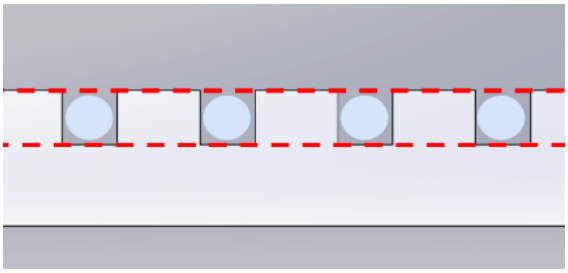
Figure 3.3 Crosslinked GelMA pattern when exposure was done at 20x.

The ratio of photomask pattern dimension to crosslinked GelMA pattern dimension was calculated. The area of crosslinked GelMA patterns was measured by ImageJ and the scales were set according to the data mentioned in the previous chapter of how many micrometers per pixel were in the microscope image. It was 0.3397 µm /pixel for 10x, 0.1645 µm /pixel for 20x and 0.08417 µm/pixel for 40x. The average ratio was 0.050 mm on the photomask per µm of the crosslinked GelMA and 0.048 mm on the photomask per µm of the spaces between crosslinked GelMA. For example, to produce circular patterns of

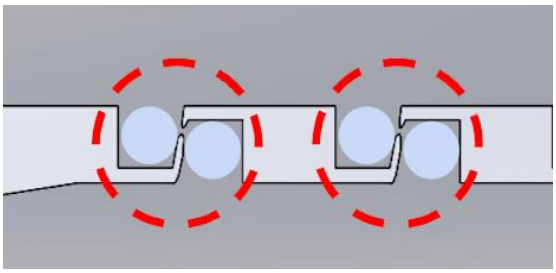
35 μm diameter with 25 μm spacing, the photomask design would be a circular pattern of 1.75 mm diameter and 1.2025 mm spacing in between.

As suggested by Li [1], the improvement would be using a photomask that contained arrays of transparent dots matching the position of the cell traps and cell pairing sites. The light would pass through the transparent dots and crosslink GelMA accordingly. This process was expected to crosslink all GelMA at the traps and pairing sites in one exposure, instead of crosslinking one at a time and repeated for 50 to 100 times on one device. The circular patterns were first designed and tested, and later the rectangular patterns were designed to avoid the accumulation of fabrication errors in each cell traps or pairing sites. The later design contained 5 rows of rectangles, and the width of the rectangles was smaller than the width of the structure to create separated compartments. Each rectangle was aligned to a row of cell traps or cell pairing sites. Since there were only 5 rows of traps or pairing sites on the device and there were usually fewer fabrication errors in the vertical direction, the results would be more accurate. Figure 3.4 A, B and C are the schematic of the cell trapping and cell pairing devices with cells in the traps and the region where GelMA crosslinked is the area surrounded by the dotted lines.

(A)



(B)



(C)

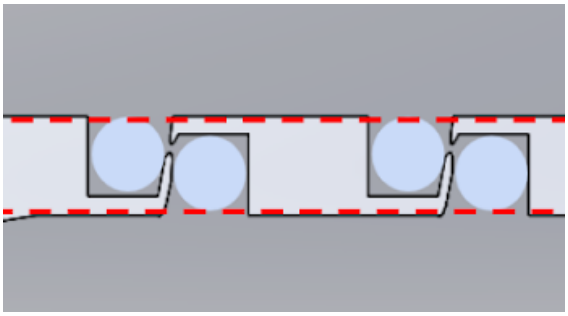


Figure 3.4 Schematic of cell trapping and cell pairing device with designed circular and rectangular GelMA crosslinked patterns.

(A)Cell trapping device with designed rectangular GelMA patterns shown in the dotted line and circular cells in the traps. (B) Cell pairing device with designed circular GelMA patterns shown in the dotted line and circular cells in the pairing sites. (C) Cell pairing device with designed rectangular GelMA patterns shown in the dotted line and circular cells in the pairing sites.

There were two final basic designs of the photomasks- circular patterns as suggested by previous work [1] and the rectangular patterns which aligned to each row. The cell trapping microfluidic device contained 20 traps in each row and 5 rows on a

device. The cell pairing microfluidic devices contained 10 pairing sites in each row and 5 rows as well. With this basic design principle for cell trapping and cell pairing devices, there were variations in the size of traps and pairing sites or variations in the horizontal and vertical spacing between the traps and pairing sites. In this thesis, the cell trapping photomask was designed for the cell trapping device with 10 μm traps, 15 μm horizontal spacing, and 40 μm vertical spacing which was the channel. Three cell pairing photomasks design, including two circular patterned and one rectangular patterned photomask, were designed for the two cell pairing devices where the pairing sites had the length of 30 μm and width of 15 μm. Both devices had the same vertical spacing of 50 μm between the rows, but the horizontal spacing was 25 μm and 55 μm. Figure 3.5 A shows the circular patterned mask design for 25 μm spacing cell pairing devices and Figure 3.5 B shows the rectangular patterned design for the cell trapping devices. There are advantages and disadvantages to both basic designs. The circular photomasks better fit the cells since they are also circular, but it is hard to create compartments at each microwell accurately due to the fluctuation of energy from the light, refraction of light along the path, diffraction of light through the photomask patterns, non-uniform light to the surface, and any other fabrication errors of the devices or GelMA. The error at each microwell would accumulate and result in large differences in the end. However, the circular cross-linked GelMA compartments were able to keep cell pairs isolated even when the structure breaks or when it is not perfectly fabricated in photolithography or soft lithography due to the high aspect ratio of the structure. The advantages of rectangular masks are the following. They can fit any device with the same vertical dimension, so there are more flexibilities on horizontal positions. Also, the GelMA patterns created are less sensitive to the errors since they don't need to

match each trap or pairing site exactly. The structures of cell trapping devices had lower aspect ratio and the microwells are too close together, therefore the photomask design was rectangular.

(A) (B)

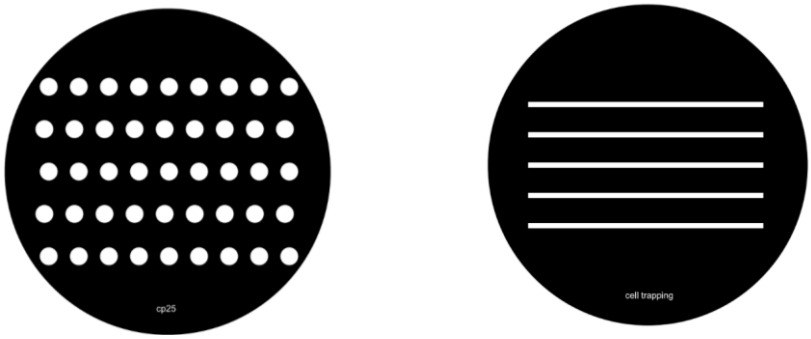


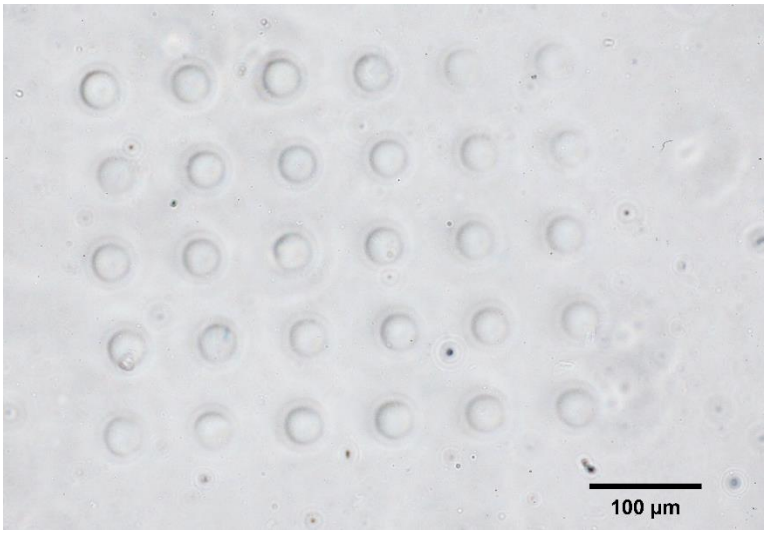
Figure 3.5 Photomask design for GelMA crosslinked patterns on the cell pairing and cell trapping devices.

(A)Photomask design for 25 µm spacing cell pairing microfluidic devices. (B) Rectangular design for 10 µm - traps cell trapping microfluidic device.

3.2 Optimization of the GelMA Processing Parameters

All three designs for cell pairing devices cannot produce all the compartments with one-time exposure. For the 25 µm spacing circular patterned and rectangular patterned masks, the first and tenth compartments on the edge were missing for the first and last row. The 55 µm spacing circular patterned mask will not fit in one-time exposure as well with only 6 columns were able to fit in one mask. Patterns created in first exposure are shown in Figure 3.6 A. The second exposure was required and achieved by aligning the remaining compartments to the existing patterns recorded on the screen. The completed crosslinked patterns are shown in Figure 3.6 B. The results from the first and second exposure of rectangular patterned photomask are shown in Figure 3.6 C and Figure 3.6 D.

(A)



(B)

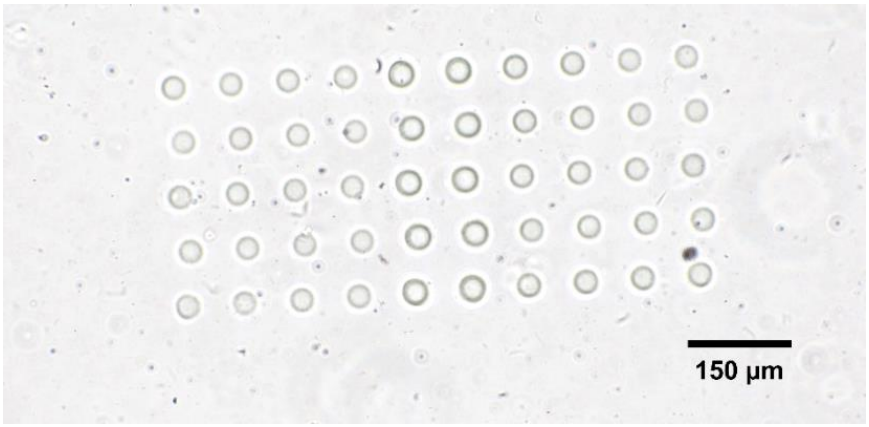
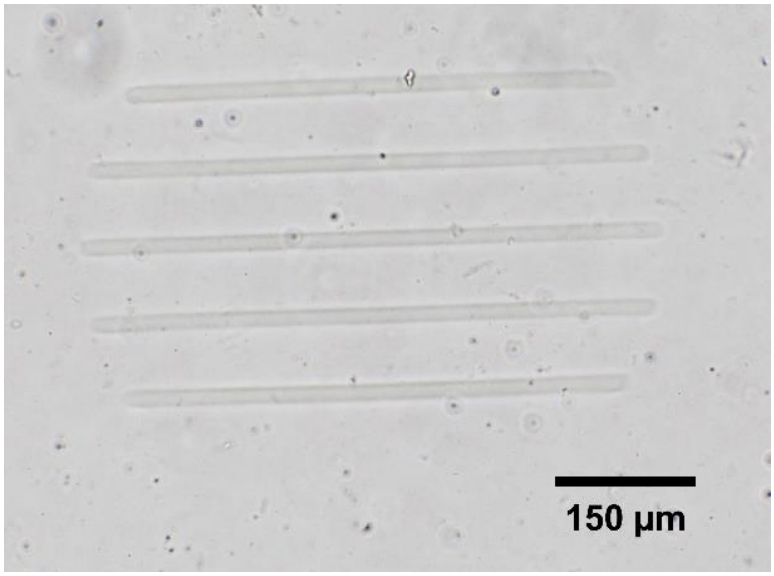


Figure 3.6 Completed patterns of crosslinked GelMA with two-time exposure.

(A)The first exposure of 55 µm spacing circular patterned mask. Only 6 columns were crosslinked. (B) Second exposure of 55 µm spacing circular patterned mask with a total of 10 columns.

(C)



(D)

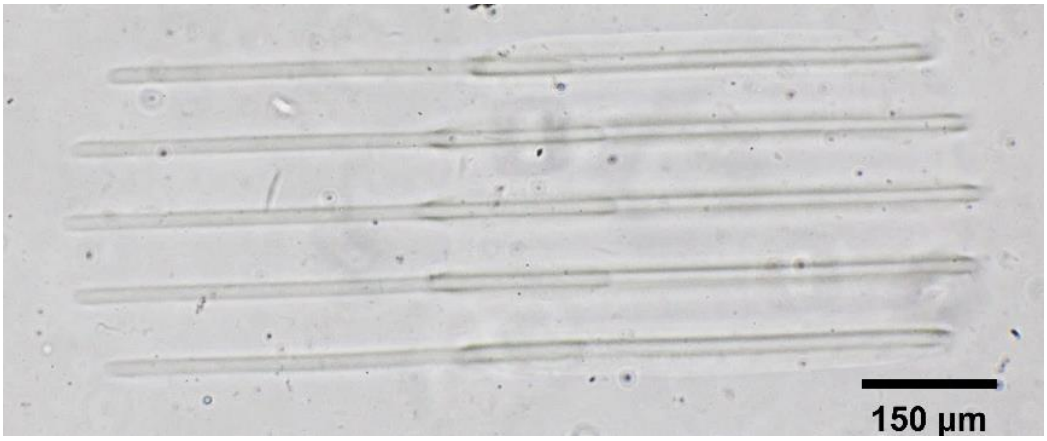


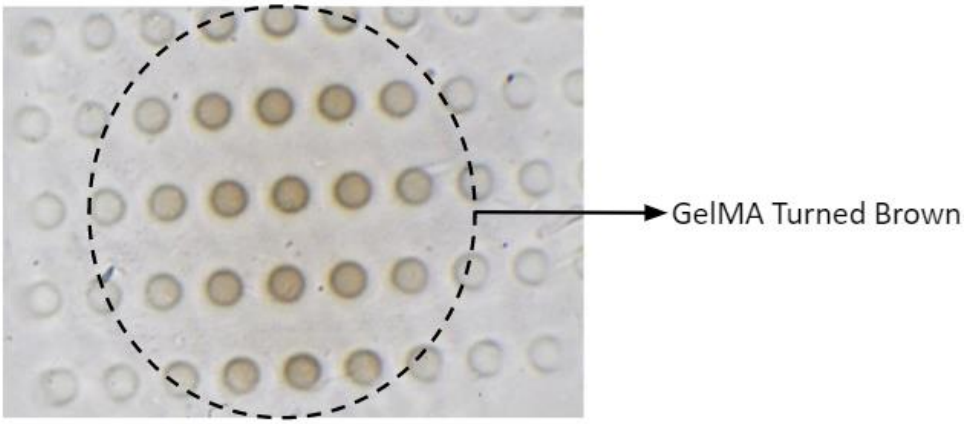
Figure 3.6 Completed patterns of crosslinked GelMA with two-time exposure.

(C) The first exposure of a rectangular patterned mask. (D) The second exposure for rectangular patterned mask connection to the first exposure.

The length of time needed to crosslink GelMA under UV light was also tested. The average exposure time was 15 seconds. As shown in Figure 3.7, the color of GelMA changed from clear to brown after excessive exposure. The length of time needed depends on the lamp intensity and it will be affected by the lifetime of the lamp. If the lamp is within the

lifetime limit of 200 hours, the range of acceptable exposure time is between 15~30 seconds.

(A)



(B)

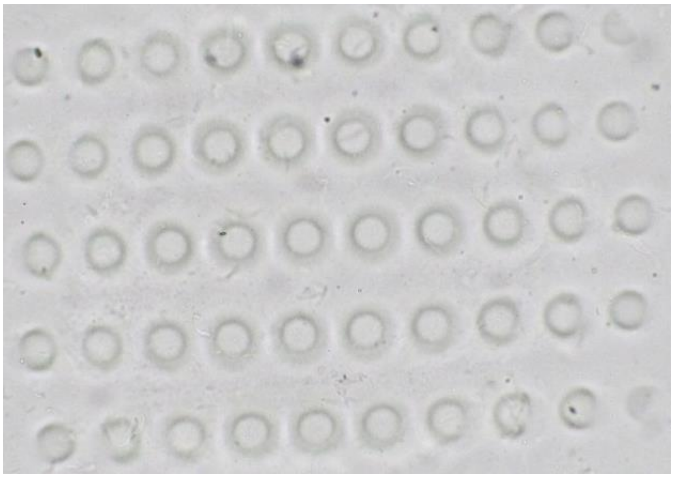


Figure 3.7 GelMA turned brown after excessive exposure.

(A) Crosslinked GelMA turned brown after excessive exposure. (B) Crosslinked GelMA without excessive exposure was clear in color.

3.3 Finalized Procedure of the New Method to Create GelMA Compartments

After all the testing, the finalized experiment procedures are the following.

1. Design the microfluidic device master molds by CAD software and fabricate it by two-layer lithography.
2. Design emulsion glass photomasks according to the design of microfluidic devices using computer-aided design (CAD) software and order the mask from the vendor.
3. Laser cut the emulsion glass photomask to 32 mm.
4. Mix PDMS and the curing agent at a ratio of 10:1 and put the mixture into the vacuum desiccator for one hour to eliminate the bubbles.
5. Pour PDMS and curing agent mixture onto the master molds and bake at 120 ℃ for 5 minutes. Leave it on a flat surface at room temperature overnight.
6. Cut out the PDMS and punch the inlet and outlet.
7. Plasma bond the PDMS structure to a clean glass slide.
8. Prepare GelMA and Irgacure 2959 mixture by adding 10% w/v GelMA solution with PBS, PBS, and 50% w/v Irgacure 2959 (with Dimethyl sulfoxide) at the ratio of 50:49:1.
9. Setup of the microscope- fully open the field iris diaphragm, place the emulsion glass photomask in the filter slider.

The first part of the experiment is to find out the position where the crosslinked GelMA patterns will be created on the device by the photomask using a testing microfluidic device with no microstructure and wider channel.

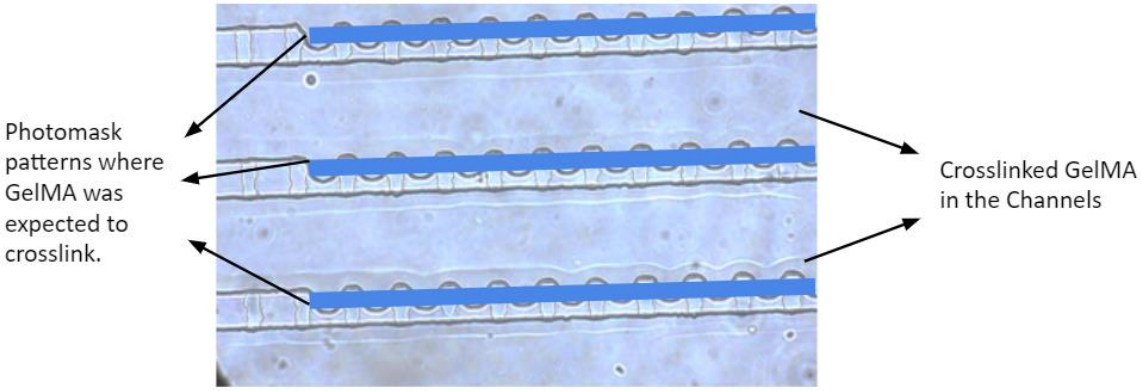
10. Inject the mixture of GelMA and Irgacure 2959 into the testing microfluidic device.
11. Find a blank space in the channel and adjust the focus using a 10x objective lens of the microscope.
12. Change it to a 40x objective lens.
13. Change to DAPI. Open the shutter for exposure for approximately 15 seconds, and no more than 30 seconds.
14. Close the shutter.
15. Markdown the patterns on the computer screen for later alignment.
16. With the positions of patterns confirmed, align the structures of actual cell trapping or cell pairing device to the position marked on the screen.
17. Repeat steps 10 to 14 as many times as needed to fully crosslink the whole device.

3.4 GelMA Photopatterning on Cell Trapping and Cell Pairing Microfluidic Devices

Although the GelMA crosslinked patterns of correct dimension could be fabricated on a testing microfluidic device using the new method, the same patterns did not appear on the actual cell trapping and cell pairing microfluidic devices. Figure 3.8 A and Figure 3.8 B show the crosslinked GelMA patterns on cell trapping and cell pairing devices when using the photomasks. GelMA crosslinked at a distance from the microstructures. Therefore, crosslinking in the traps, where were closely surrounded by the structures, were not successful. One way to crosslink the GelMA in the traps was to expose it to the UV light without a photomask. GelMA crosslinked in a whole circle within the range of light source

which contains the channels as well (Figure 3.8 C). However, it did not crosslink the GelMA in every trap. This problem is possible to be solved when the cells are in the traps. According to previous work [2], the cells in the traps connected to the crosslinked GelMA in the channel and completed the compartments. When the photomasks were not used, the problem of trapping the whole channel could only be solved if the field iris diaphragm is closed and limits the range of light like the previous method [1].

(A)



(B)

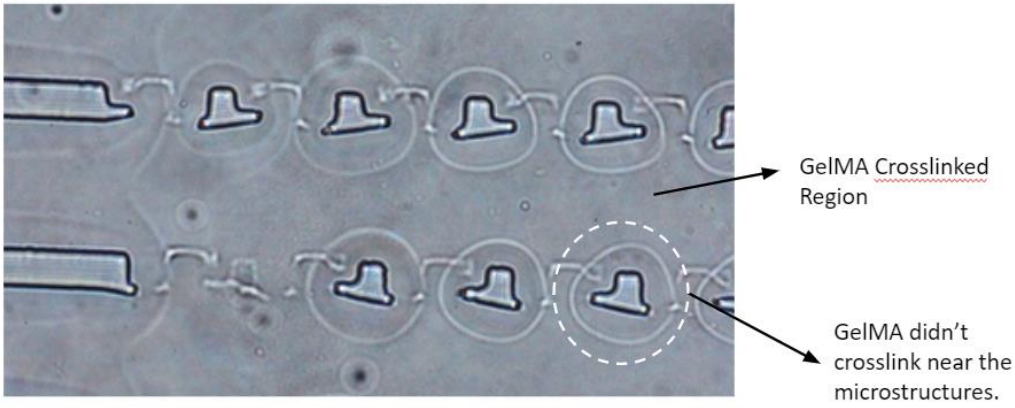


Figure 3.8 GelMA on the cell trapping and cell pairing microfluidic devices.

(A)GelMA crosslinked in the channels of the cell trapping microfluidic device with the photomask. (B) GelMA crosslinked around the microstructures of the cell pairing microfluidic device with the photomask.

(C)

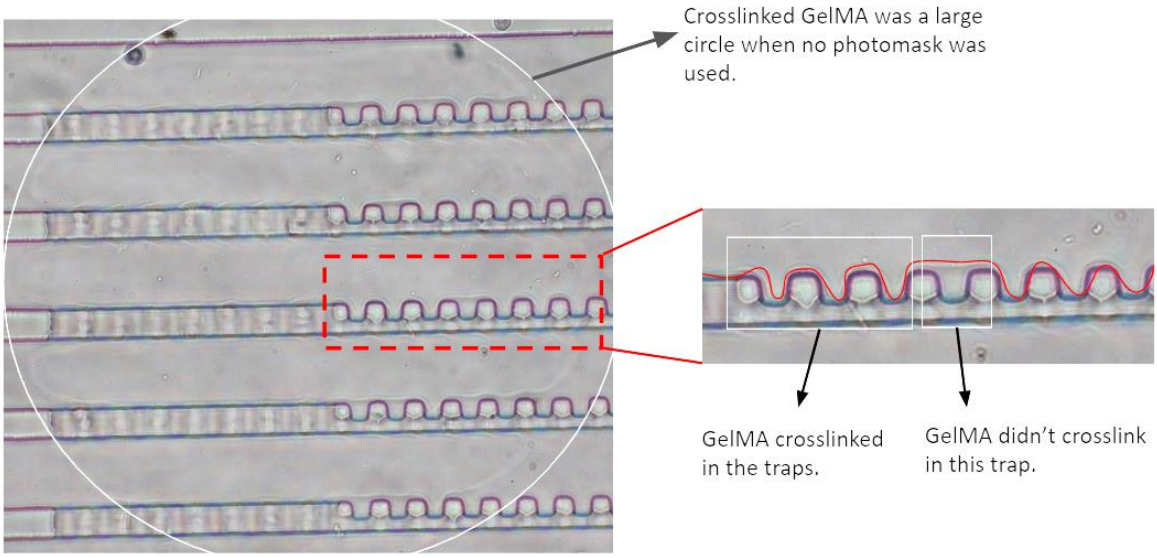


Figure 3.8 GelMA on the cell trapping and cell pairing devices.

(C) GelMA crosslinked a whole circle shown in green in the middle while not using the photomask to block certain patterns of UV light on the cell trapping device. Some of the traps of the cell trapping microfluidic device contained crosslinked GelMA and some did not. The figure on the right is the third row of the figure on the left. There were six traps containing crosslinked GelMA and one trap didn't contain crosslinked GelMA.

The possible causes for unsuccessful photopatterning of GelMA were analyzed by comparing the following methods and results. First, the previous method [1] was compared with the new method. The similarity between the two methods was that the range of light was limited to a small circle around the pairing site. The differences were the physical block of the light and the number of circles nearby. The physical block was the field iris diaphragm of the microscope for the previous method, but it was the emulsion glass photomask for the new method. There were arrays of circles on the photomask close to each other, but there was only one circle in the middle for the previous method. The possible cause of the error was that the light going through the clear pattern of photomask diffracted and when it reaches PDMS, it was at an angle. The bent light made the refraction

of light through PDMS structures more significant. The circular patterns were not far apart and the light passing through each circle went in an undesired direction and affected each other. From the experiment results, GelMA in the channels were easier to be crosslinked than GelMA in the traps in both cases. The previous method worked in a way to crosslink a small part of the channel above and below the pairing site by closing the field iris diaphragm. GelMA in the traps was crosslinked occasionally and the crosslinked GelMA in the channels connects to the cells in the traps. With the help of the cells, the GelMA compartments were successfully fabricated. There was a wider range of light for the new method when the field iris diaphragm was fully opened. The light diffracted and refracted through the photomask and PDMS, so it was no longer limited by the patterns of the photomask. GelMA in all the channels was crosslinked.

Comparing the testing and actual devices, there was no structure affecting the direction of light on the testing device. For the same possible reason as above, the light diffracted through the small clear patterns of photomasks and the bent light refracted when passing through the PDMS microstructures on cell trapping and cell pairing microfluidic devices. The testing device only contained a thin top layer of PDMS on the top and it was relatively uniform than the complex PDMS microstructures on the actual device cell trapping and cell pairing microfluidic devices.

CHAPTER 4: Conclusion and Future Research

4.1 Conclusion

With the GelMA compartments, the target cell and cell pairs on the cell trapping and cell pairing microfluidic devices can be cultured and observed for a long period of time. The current method has improved from the previous method of creating the GelMA compartments one at a time [1]. It can create arrays of 50 to 100 GelMA compartments matching the design of the cell trapping and cell pairing devices within 30 seconds. Through the experiments done on the blank testing microfluidic devices, the experimental setup, photomask design, and other parameters of the process were finalized. An inverted microscope was used as the UV light source and observing tool. Other materials required include GelMA and Irgacure 2959 mixture and photomasks. GelMA can be crosslinked with the UV light exposure in 15 to 30 seconds. In order to produce more patterns or larger areas of patterns, multiple exposures are required. Considering the resolution of the crosslinked GelMA, the efficiency of the procedure and the size of the microfluidic device, the exposure was done when using a 40x objective lens. With some small-scale adjustments on the mask pattern or dimension, the method can be applied to different designs of microfluidic devices.

Although the goal was to create the crosslinked GelMA patterns on the cell trapping and cell pairing microfluidic devices, the method was not yet successfully performed on the devices. Two sets of results were compared and analyzed. First, the results of the new method and the previous method were discussed. Second, the results on the testing devices

and actual cell trapping and cell pairing microfluidic devices were discussed. A possible cause of the error was that the PDMS microstructures refract the light into an unpredictable direction and crosslinked GelMA in the channels.

4.2 Future Research

Future improvement on the method would be solving the refraction issue of UV light as it passes through the PDMS structures or a method that uses the GelMA as the material for microstructure directly without complex PDMS structures. The refractive index for PDMS is around 1.4 and it increases as the wavelength decreases [18]. In order to crosslink GelMA, UV light is necessary which has lower wavelengths that increase the refractive index for PDMS. If the refractive index can be lowered for PDMS or use other materials with a lower refractive index, the issue of refraction will have less effect. Another way to eliminate the effect of light refraction through PDMS is to use only GelMA for the microstructures. Examples of using GelMA directly as the microstructures are described in [16] and [17] that the complex 3D GelMA structures were fabricated by laser and dynamic projection stereolithography layer-by-layer.

REFERENCES

[1] X. Li, “Microfluidic Single-Cell Analysis from Phenotype to Genotype,” Ph.D. dissertation, Biomedical Eng., Univ. California, Irvine, Irvine, CA, 2019.

[2] K. Yue, *et al*., “Synthesis, properties, and biomedical applications of gelatin methacryloyl (GelMA) hydrogels,” Biomaterials, vol. 73, pp. 254-271, Dec, 2015 019.

[3] M. J. Madou, “Scaling Law,” in Fundamentals of Microfabrication and Nanotechnology Volume III- From MEMS to Bio-MEMS and Bio-NEMS Manufacturing Techniques and Applications, Bocaa Raton, FL, USA: CRC Press, 2011, ch. 7, pp. 359–386.

[4] G. M. Whitesides, The origins and the future of microfluidics. Nature 442, 368-373. Nature. 442. 368-73. 10.1038/nature05058.

[5] E. Sackmann, A. Fulton, D. Beebe, “The present and future role of microfluidics in biomedical research.” Nature. 507. 2014. 181-9. 10.1038/nature13118.

[6] Luo T, *et al*., “Microfluidic Single-Cell Manipulation and Analysis: Methods and Applications.” *Micromachines* vol. 10,2 104. 1 Feb. 2019, doi:10.3390/mi10020104

[7] Collins, D.J., *et al*., "The Poisson distribution and beyond: Methods for microfluidic droplet production and single cell encapsulation." Lab Chip 2015, 15, 3439–3459.

[8] B. M. Taff, J. Voldman, "A scalable addressable positive-dielectrophoretic cell-sorting array." Anal. Chem. 2005, 77, 7976–7983.

[9] K. Zhang, *et al*., "Single-cell isolation by a modular single-cell pipette for RNA-sequencing." Lab Chip 2016, 16, 4742–4748.

[10] L. Feng, P. Di, F. Arai, “High-precision motion of magnetic microrobot with ultrasonic levitation for 3-D rotation of single oocyte.” Int. J. Robot. Res. 2016, 35, 1445–1458.

[11] D.J. Collins, *et al*., “Two-dimensional single-cell patterning with one cell per well driven by surface acoustic waves.” Nat. Commun. 2015, 6, 8686.

[12] D.R. Gossett, *et al*., “Hydrodynamic stretching of single cells for large population mechanical phenotyping.” Proc. Natl. Acad. Sci. USA 2012, 109, 7630–7635.

[13] S. Hu, D. Sun, "Transportation of biological cells with robot-tweezer manipulation system." In Proceedings of the 2011 IEEE International Conference on Robotics and Automation, Shanghai, China, 9–13 May 2011; pp. 5997–6002.

[14] J. Gao, X. Yin, Z. Fang, "Integration of single cell injection, cell lysis, separation and detection of intracellular constituents on a microfluidic chip." Lab Chip 2004, 4, 47–52.

[15] R. Huys, *et al.,* "Single-cell recording and stimulation with a 16k micro-nail electrode array integrated on a 0.18 μm CMOS chip." Lab Chip 2012, 12, 1274–1280.

[16] A. Ovsianikov, *et al.,* "Laser Fabrication of 3D Gelatin Scaffolds for the Generation of Bioartificial Tissues." *Materials (Basel, Switzerland)* vol. 4,1 288-299. 19 Jan. 2011, doi:10.3390/ma4010288

[17] P. Soman, *et al.,* "Digital microfabrication of user-defined 3D microstructures in cell-laden hydrogels." *Biotechnology and bioengineering* vol. 110,11 (2013): 3038-47. doi:10.1002/bit.24957

[18] F. Schneider, *et al.,* "Process and material properties of polydimethylsiloxane (PDMS) for Optical MEMS.", Sensors and Actuators A: Physical, vol. 151, Issue 2, pp 95-99, (2009) ISSN 0924-4247, https://doi.org/10.1016/j.sna.2009.01.026.