DEVELOPMENT OF GEL DROPLET MICROFLUIDIC SYSTEM FOR HIGH THROUGHPUT MICROBIAL SCREENING

A Thesis

by

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ABSTRACT

Over the last few decades, droplet based microfluidics has received great attention and it is growing rapidly with interdisciplinary fields, such as biomedical, physics, chemical engineering, tissue engineering and even therapeutic area. Droplet based microfluidics offers uncountable potential in its applications ranging from a developing analytical system to precise controlling of the content inside the droplet. One of the most highlighted advantages of droplet based microfluidics is the capability of cell screening in high throughput manner, which results in significant reductions in cost and discovering new medicine or curing technology.

Nowadays, culturing encapsulated cell in 3D environment has been required and performed. Although conventional 2D culturing has simplicity of platform but because 3D environment has the capability of providing high throughput biological assays and an environment similar to native biological complexes, it was chosen for this study. Furthermore, the field of biomedical or biomaterials prefer to improve that 3D environment to be much closer to genuine systems. From this respect, hydrogels have been proven and replaced as a useful platform for 3D cell culture applications in microfluidics and cell laden hydrogel droplet is one of the most popular application with their flexibility similar to natural tissue and mild gelation method. In this thesis, we studied two different types of hydrogel droplets and developed a strategy for the microbial co-culture platform from the 'platform' perspective. This thesis focuses on the microfabrication to pattern silicon wafer

mold for the mass production and creating polydimethylsiloxane (PDMS) devices. With this transparent hydrophobic material, hydrogel droplet generation, on-chip cross linking and manipulation could be possible.

DEDICATION

To my lovely wife Sulki, parents, parents-in-law, sister and brother-in-law for their unconditional love, support and trust.

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NOMENCLATURE

PDMS	Polydimethylsiloxane
PEG	Polyethylene glycol
PEG-NB	PEG-norbornene
PAC	Photo Active Component
DI water	De-Ionized water

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1. INTRODUCTION

1.1 Objective and motivation

Over the last few decades, droplet based microfluidics has been received a great attention and it is growing rapidly with interdisciplinary fields, such as biomedical, physics, chemical engineering, tissue engineering and even therapeutic area [1]. Droplet based microfluidics offers uncountable potential in its applications range from a developing analytical system to precise controlling of the content inside the droplet. One of the most highlighted advantage of droplet based microfluidics is the capability of cell screening in high throughput manner, which result in significant reductions in cost and also discovering new medicine or curing technology [2] [3]. Nowadays, culturing encapsulated cell in 3D environment has been required and performed although conventional 2D culturing has simplicity of platform due to their capability of providing mechanical environment similar to native biological complexes [4] [5]. Furthermore, the field of biomedical or biomaterials prefer to improve those 3D environments to be much closer to genuine systems. From this respect, hydrogels have been proven and replaced as a useful material for 3D cell culture applications [6].

Hydrogels are biocompatible and biodegradable hydrophilic material that consisting of network of polymer chains. Hydrogel also have controllability of their porosity size. It makes us to load any size of molecules and diffuse them to targeted position in biological system. In addition to this, hydrogels can also be deformed, conformed by artificial ways

or conditions and due to their adhesive characteristic, they have a tremendous benefit in drug delivery applications. Cell laden hydrogel is also one of the most popular application due to their flexibility similar to natural tissue and mild gelation method. Despite these advantages and desirable properties, there are some disadvantages in using hydrogel. Their biodegradability is not always desirable since some applications require drug traveling during certain time period or location. Thus, researchers in those areas want to have specific hydrogel depending on their work [7] [8]. Different types of hydrogel can be prepared from nature and even artificially and utilized depending on specific application. Therefore, there are many gelation methods, for example, ionic cross-linking, thermal cross-linking, and photo cross-linking. Furthermore, cells encapsulated micro hydrogel are still suffer from their environment. Cell to cell communication and nutrients exchange are restricted due to their limited diffusion and distances between droplets [9]. Also, since the membrane of hydrogel is vulnerable by mechanical shear force, hydrogel is not always appropriate material on certain application. As the figure 1.1 illustrated, the motivation of this work is by utilizing micro scaled fluidic techniques, we could be able to create microbial co-culture platform by using hydrogel droplet, in which cell to cell communication between hydrogel can be happen. Ultimately, screening different types of cell encapsulated in gel droplet on-chip system. Still we have lots of works need to be done to make 3D hydrogel platform. Here in this work, we focused on the fabrication of 2-dimensional polymer based droplet community for further cell research. In the following chapters, details will be explained

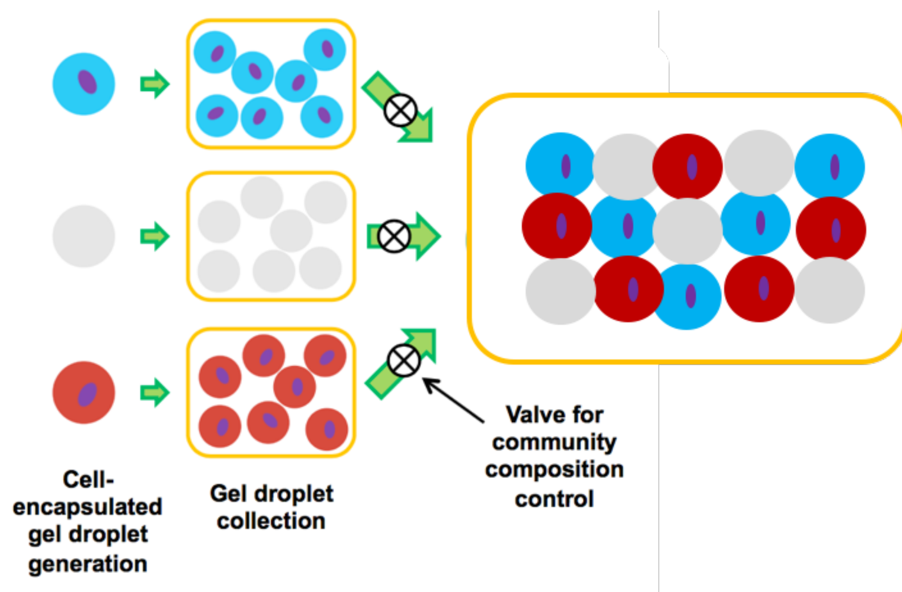

Figure 1.1: Hydrogel based microbial co-culture platform

1.2 Gel materials

Hydrogels are the most favorable material in biomedical engineering area and the most significant factor that researchers should consider when they treat hydrogel is the content material. Since cells for culturing in micro scaled droplet are sensitive in terms of cell growth rate and cell viability, many different types of hydrogel should be used depending on each purpose and currently there is no material that satisfy all of the requirements at once [10] [11] [12]. Here, we overview different type of hydrogel material.

1.2.1 Protein based hydrogel

Collagen is the most abundant and major component of human body, like skin and bone. They have been characterized with many different types of triple helices that can form an aggregate with neighboring collagen molecules. Although there are many collagens, collagen type I is one of the most commonly studied material for biomedical and tissue engineering due to their abundance, good biodegradability, low immunogenicity and cell-binding ability. However, the poor mechanical property and rapid degradation rate of non-cross-linked collagen impede robust tissue regeneration. Increasing the concentration of collagen might have been an alternative to make it mechanically stronger, but cell migration and nutrients diffusion will be damaged. Therefore, many different strategies for cross linking collagen have been developed so far to enhance its robustness and mechanical property. The most common method for polymerization of collagen is thermally driven method. However, it requires at least 30 minutes to increase temperature. Thus, it occasionally needs additional incubation process after generation of collagen based micro scaled droplet.

Fibrins is an important component of blood clotting and it regulates would healing. Fibrin is a polymer-like material from precursor plasma, called fibrinogen. If patient has a vascular injury, the enzyme thrombin will be released and cleaves the peptide fragment from fibrinogen to form fibrin peptides that can generates fibrils, which consequently gather into a fibrin network that is cross-linked by addition of transglutaminase factor XIII. The polymerization time is a critical factor of properties of microstructure, such as fiber

size or porosity. For example, high degree of thrombin concentration makes more rapid gelation, allowing a tighter network with less porosity. Because thrombin has a sodium cation binding side where controls its activation, addition of sodium chloride can increase the polymerization time unto several minutes. In addition to its great biocompatibility, biodegradability, and cell adhesiveness, the mechanical characteristics of fibrin hydrogel can be turned by varying the initial concentrations of fibrinogen and thrombin. One of the most favorable advantage of fibrin is that it can form a temporary matrix, which serves as scaffold that promotes the penetration of cells until the wounded tissue is regenerated. This indicates that fibrin clotting is natural with proper cell-mediated degradation mechanism to balance the time of regeneration and biological environment remodeling, which is ideal situation with synthetic materials.

Gelatin is denatured form of collagen, consisting of a wide range of amino acid and it is thermo-responsive, undergoing reversible sol-gel transition by lowering the temperature of water based polymer solution below 37 °C, which is impractical to use directly as an injectable in situ curable gel. However, this disadvantage can overcome by adding methacrylate groups to the side chains of gelatin, resulting in gelation. An additional advantage of this way is that the mechanical and chemical characteristics can be modified by adjusting parameters in the methacrylate and photo cross linking processes.

1.2.2 Natural polysaccharides

Agarose is extracted after a series of purification and homogenization process from a group of rhodophyceae, including gelidium and gravilaria. It is a neutral, bio-inert consisting of β-D-galactopyranose and 3,6-anhydro-alpha-L-galactopyranose. Agarose is a polymer and its solution undergo a sol-gel transition when they are cooled. Similar to gelatin, the gelation of agarose is thermally reversible. Thus, they can be solidified under 37 °C, which results in the aggregation of double helices. Agarose micro droplet also can be formed by cooling down the temperature. However, the gelation time and temperature depends on their concentration and molecular weight, so there are many different types of commercialized agarose. Even though agarose does not provide adhesion to cell, it can be supplemented with adhesive molecules, such as fibrinogen or RGD soluble peptide. The biggest issue with agarose is that they are not biodegradable. It can only be degraded by certain bacteria. Therefore, the poor biodegradability inhibits the natural regeneration or repair process in vivo.

Chitosan is a polysaccharide consisting of β-1, 4-linked D-glucosamine and N-acetyl-D-glucosamine unit from chitin. Chitin is chemically similar structure to glycosaminoglycans. Different from agarose, it has excellent biodegradability by enzymatic method. It is also biocompatible, antibacterial characteristics, and low toxicity. Moreover, its hydrophilic property provides cell adhesion and proliferation. Chitosan hydrogel can be generated by thermally induced gelation or pH concentration. The chitosan hydrogel can be mixed with other polymer to enhance their mechanical properties. Its amine group allows

the conjugation of molecules to adjust their biological properties. For example, Fan et al, utilized non-metal click chemistry to form an oxanorbornadiene-functionalized chitosan and azido-functionalized hyaluronan composite hydrogel, its gelation and microstructure show its properties such as equilibrium swelling and degradation kinetics. However, one of the biggest problem in using chitosan is that it is difficult to get highly purification level, medical-using chitosan. Therefore, impurities can affect their de-polymerizaion rate and biological responses.

Hyaluronic acid consisting of alternating units of the disaccharide β-1,4-D-glucuronic acid-β- 1,3-N-acetyl-D-glucosamine, is a non-sulfated glycosaminoglycan that is found in most connective problems. It is significantly important in wound healing, cellular signaling, morphogenesis, and organization of the ECM. Also, hyaluronic acid interacts with cell-surface receptors affecting tissue organization, stimulates the production of collagen II and promotes cell proliferation. Due to their good biocompatibility and non-immunogenicity, it is a biologically relevant material for generating hydrogels. Hyaluronic acid-based hydrogels can be formed using a wide range of gelation methods, including photo cross linking, thermally induced cross linking, and covalent cross linking. Among these methods, photo polymerization is the most common strategy for generation of hyaluronic acid hydrogels. For example, the carboxylic acids on the hyaluronic acid backbone can easily be modified with methacrylic anhydride to make methacrylated hyaluronic acid, whose network can be controlled by varying the wavelength of UV exposure, molecular weight, concentration, and number of reactive groups.

1.2.3 Synthetic polymers

PEG (polyethylene glycol) is a biocompatible and biodegradable hydrophilic polymeric network chains comprising a PEG diol with two hydroxyl end groups that can be changed into other groups to make hydrogels with different mechanical and chemical properties. Even though PEG had a degradability, it is poor and negligible and it is also resistant to cell adhesion, which is not ideal for hydrogel droplet based applications for cell encapsulation. To increase the advantages, enzymatic peptide sequences can be used and injected into PEG hydrogels to let its degradation rate slow down and make them responsive to cells. The most common gelation method with PEG hydrogel is photo cross linking, which has a good in situ gelation at physiological temperature and pH. Furthermore, the light intensity can be minimized by choosing appropriate concentration and photo initiator chemistry, resulting in PEG gelation process more cell friendly.

1.3 Gelation of hydrogel

The encapsulation of cells in hydrogel droplet starts by suspending cells in water based precursor of a hydrogel, which is liquid phase. Then, the suspension experiences the phase transition from flowing to non-flowing phase by physical, chemical processes. All of those processes should be biocompatible with cell survival and should require minimal stress to cells, which means that the environment surrounding cells should be close to biological conditions as possible as it can. Some cases don't satisfy those requirements, such as

photo-cross linking. Thus, it requires minimal of exposure and toxicity than other methods [12]. In this study, we will treat the physical gelation and chemical gelation.

1.3.1 Physical gelation

Physical gelation can be happened by environmental stimulations, for example, pH changes, transition of temperature and secondary reactions such as electrostatic and hydrophobic bonding. Even though the gelation can be happened under moderate conditions, the physical gelation network basically has low mechanical resistance and strength and has bad stability in biological environments. There is several physical gelation, such as electrostatic interaction, thermal gelation, and hydrogen bonding.

As figure 1.2 illustrated, the most common physical gelation method is electrostatic interaction, which can be carried out between polymers and molecules or two polymers, having opposite charges. The most famous example of the former method would be the cross-linking of alginate and calcium ions, which react with the carboxylic acid group of alginate. An example of the second method would be ionic peptide bonding with positively and negatively charged units, which can self-assemble into gelled chains.

The physical gelation can also be performed by pH changes. For example, acetic acid can be used to release the calcium ion from calcium and alginate mixed solution, allowing gelation. However, the change of pH must not be radical and should keep certain range so that the cell viability can maintain its level.

Another strategy of physical gelatin is thermal gelation, which can also be called 'sol-

gel transition'. As an example of this, the gel precursor solutions are emulsified at about 37 °C, then their droplets are cooled down to critical temperature, at which induce the gelation. This method allows to expect biocompatibility because the toxic agent can be died at this condition accept for the gel droplet precursor. However, this precursor should be kept at this condition that is higher than gelation temperature.

The last strategy of physical gelation is hydrogen bonding. It requires a mixed solution of more than two polymers that having capability of hydrogen bonding. This mixture has more viscous property than a single polymer. Due to the absence of chemical agent, it has great biocompatibility. However, they may be degraded quickly if they meet water based solution because their bonding is relatively weak.

1.3.2 Chemical gelation

Unlike the physical hydrogels, chemical gelled hydrogels have a great stability, durability and superior mechanical properties. However, using chemical cross-linking initiator cause cytotoxicity and undesired interactions between cells and proteins. Several common gelation mechanisms are described here, such as Michael addition, enzymatic reactions, and photo polymerization.

When hydrogels undergo photo cross-linking, photo-initiator stimulates a liquid monomer that can be a covalently cross-linked hydrogel, which is mechanically strong and stable. This polymerization not only can be conducted in a short time at room temperature, but can also be controlled. However, this gelation method can affect the cell by contacting

with released radicals. Thus, the concentration of photo-initiator, type of photo-initiator, light intensity and total exposure energy and time should be properly tuned to guarantee high cell viability.

Another example of chemical gelation is Michael addition, thiol-Micheal addition, in which a nucleophilic thiolate responses with electrophile. The thiol-Michael addition needs only small number of catalysts without heat or light, which provide less degradation products. However, the one problem on this strategy is that insufficient mixing of reagent will cause non-uniform gelation, which results in inconsistent cell reactions. Thus, the balance of mixing reagents and gelation rate is necessary.

Lastly, enzymatic gelation allows more physiologically and biologically chemical cross-linking than other strategies since enzymes in using hydrogel cross-linking are much more responsible and compatible for biological reactions that occurs in human body. The most commonly used enzyme for this strategy is transglutaminase, which plays a role in forming fibrin clots during wounded spot healing. This provides high and stable bonding. The enzymatic cross-linking also can prevent undesired side reactions and toxicity due to their high substrate specificity.

1.4 Droplet based microfluidics

During two decades, there has been an increase in microfluidics and its tools as well [13]. Microfluidics technology treats a broad range of fields from biology to electrical, biomedical engineering. This technology has been related from tissue regeneration to

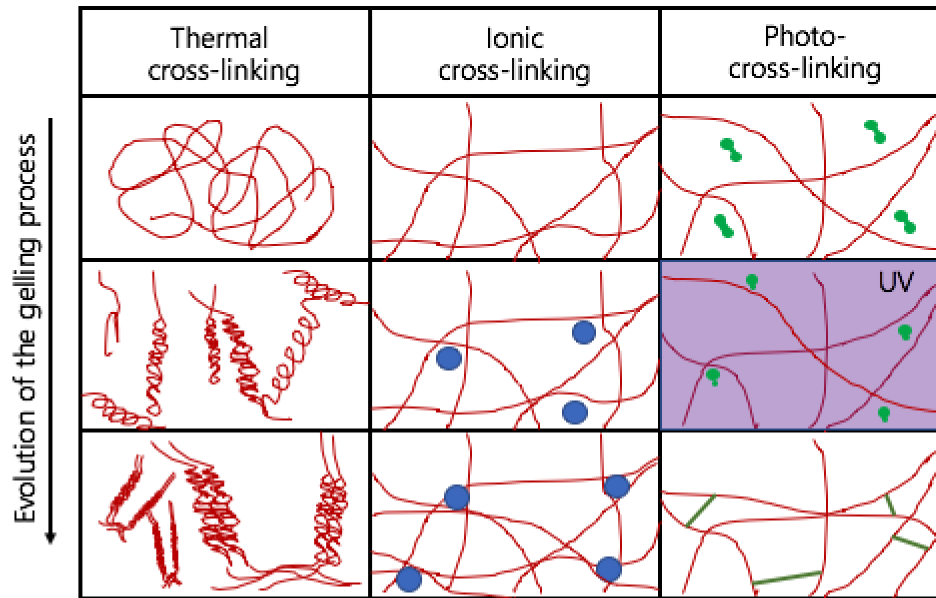

Figure 1.2: Commonly used gelation mechanisms

point-of-care medical device. Microfluidic technology has a great potential since it provides typical laboratory systems with fluidic reagents, which can be significantly reduced from milliliters to micrometers or nanometers scale. One of the most promising subcategory of microfluidics is droplet based microfluidics [14] [15].

Droplet based microfluidics concentrates on generating discrete volumes with the use of immiscible phases while typical microfluidics focus on continuous flow system. Microfluidic systems are tuned by the low-Reynolds number flow which describes that all fluid phases should maintain laminar flow regime. Laminar flow regime also allows for creating sophisticated concentration gradients that have been utilized in the cell migration [16]. Unlike typical continuous flow systems, droplet based microfluidics capable of per-

forming a huge number of fluidic reactions without increasing device size or design.

The most powerful advantage of droplet based microfluidic system is the formation of uniform sized, shaped droplets by tuning geometry of device or fluids velocity. There are a variety of droplet generation techniques, which have been already used for a long time. In order to ensure uniform sized, shaped droplet generation, proper droplet generation technique should be accompanied. Researchers have developed many droplet generation technique, such as T-junction and flow-focusing. In the T-junction area, the continuous phase and dispersed phase will intersect perpendicularly. They form an interface at the T-junction and the tip of dispersed phase starts to enter into the main channel. As the shear forces by continuous phase push the dispersed phase, the pressure gradient causes the tip of dispersed phase can be elongated until the neck part of dispersed phase become thinner and eventually breaks the stream. In the flow-focusing configuration, the symmetric design is employed and symmetric shearing force by continuous phase will affect the dispersed phase. The crucial point of flow-focusing is focused shear forces, which breaks the dispersed phase, consequently dispersed phase can be chopped. Thus, droplets are generated. The size of droplets can be controlled by changing fluid flow rates, the channel geometry or relative viscosity between two phases.

2. PEG HYDROGEL DROPLET FOR GEL SLAB FORMATION

2.1 PEG preparation and its polymerization

A cytocompatible photo clickable PEG hydrogel has been employed for the generation of droplet and its slab formation. It consists of PEG-dithiol and 8-arm PEG-norbornene (PEG-NB). The PEG-dithiol 83ul and PEG-NB 250ul are diluted in PBS to be combined with a cytocompatible photo initiator (LAP) together. Once UV light, whose wavelength is 365nm, irradiates all the components, LAP starts to form radicals and then, all components will be cross linked. The illustration of the PEG cross-linking, experiment setup, light intensity verification and bulk gelation are dipicted in figure 2.1, 2.2, 2.3 and 2.4.

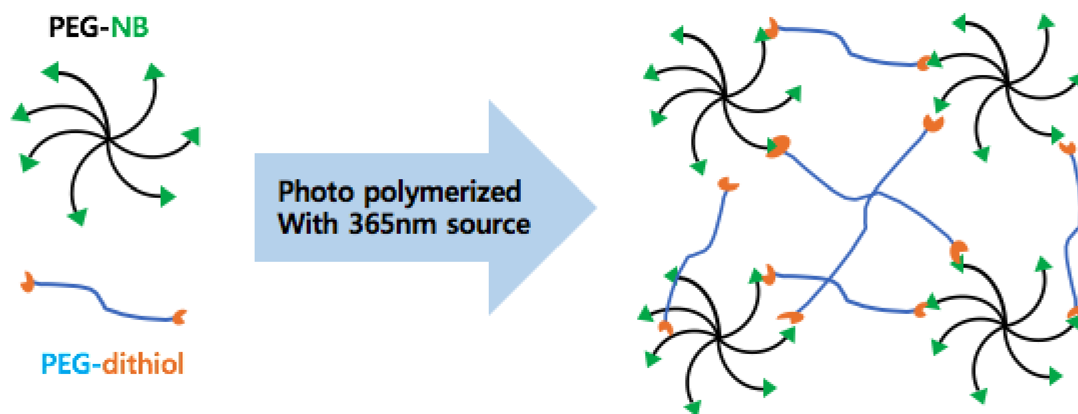

Figure 2.1: PEG hydrogel polymerization process

The bulk gelation test has been conducted under 10mW/cm2 light intensity for 30seconds. The total energy, needed for the gelation is 300mJ. The UV light intensity has been confirmed by checking the intensity value over time.

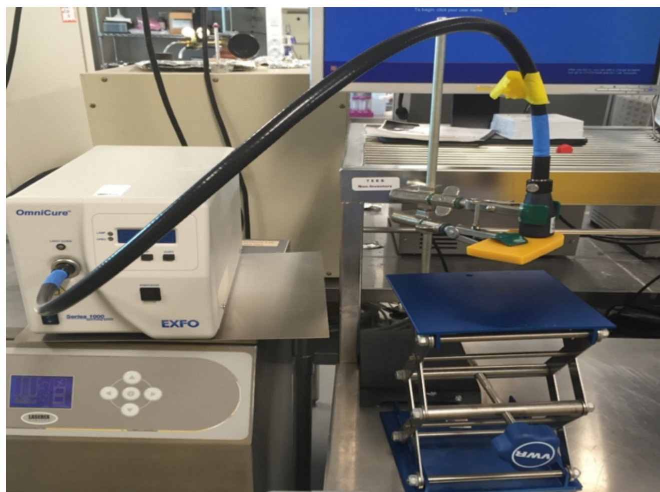

Figure 2.2: Photo cross linking device setup

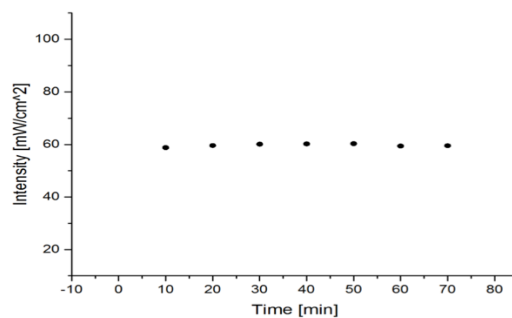

Figure 2.3: UV light intensity verification

Figure 2.4: The bulk photo cross linking test

2.2 PEG hydrogel droplet generation

The PEG hydrogel droplets are generated at T-junction droplet generator channel. The fluorinated oil (novec 7500) with 1% of PFPE-PEG type surfactant is introduced from the horizontal channel as a continuous phase and the PEG precursor solution is introduced from the perpendicular channel as a dispersed phase. Once they are meet, the continuous phase will chop the dispersed phase as it forms a plug, which is surrounded by oil. The oil with surfactant allows to have stable circular droplet morphology. However, the surfactant and oil should be removed consequently since the surfactant and oil cause side effect and are harmful when it comes to the cell culture. At the end of the horizontal channel, the plugs will change their shape to the spherical shape. The droplet size will be controlled by changing flow rate. The droplet size will be decreased if the continuous phase is increased while the droplet size will be increased if the dispersed phase is increased. At this time,

the targeted droplet size was 80um and the flow rate condition was 200ul/hour for continuous phase and 180ul/hour for dispersed phase. The T-junction mask design and droplet generation are illustrated in figure 2.5 and 2.6.

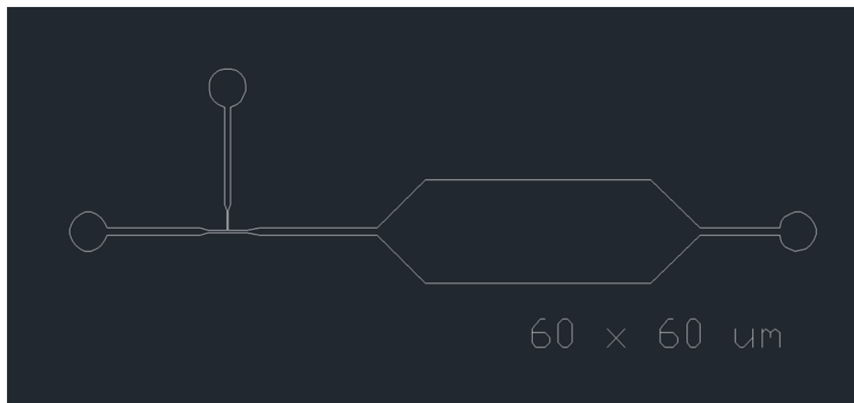

Figure 2.5: Droplet generator

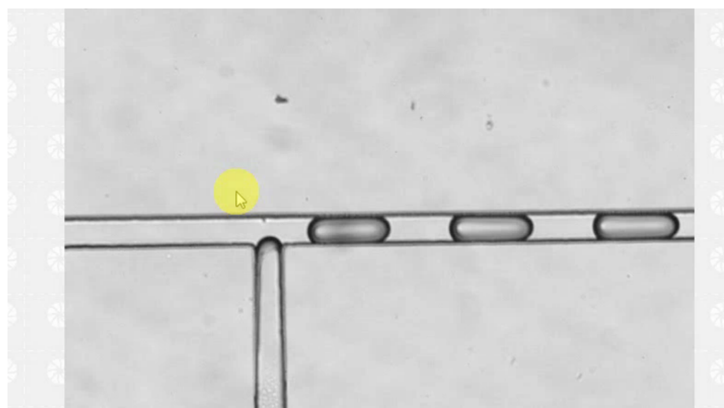

Figure 2.6: Droplet generation

2.3 Off-chip gelation and washing of PEG hydrogel

The PEG hydrogel droplets are generated and collected in micro tube for 2hours as it can be seen in figure 2.7. After the generation and collection, all droplets are transferred to well-plate for off-chip polymerization process by pipet.

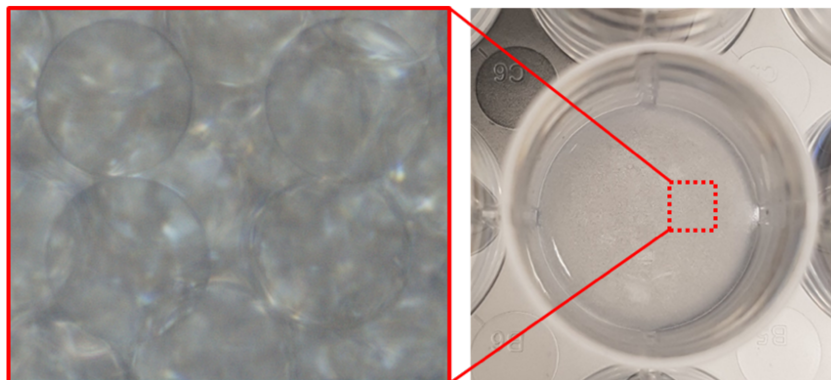

Figure 2.7: Collected PEG hydrogel droplets in well-plate

After that, as it can be seen in figure 2.8, the well-plate is placed under the UV light fiber. The gelation condition was same as the bulk gelation process, which is 10mW/cm2 for 30seconds. The UV light intensity can be controlled by changing the distance between plate and UV fiber and when the irradiation, scattering and reflection should be considered.

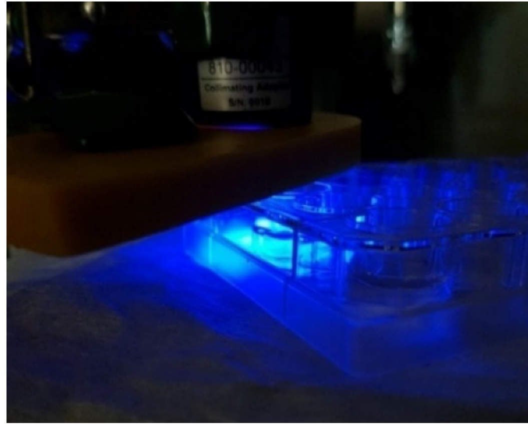

Figure 2.8: PEG hydrogel droplet polymerization

The cross-linked droplets are transferred from well-plate to strainer, which has 40um porosity. Because the droplet size is 80um, droplets cannot penetrate or leak through the strainer. The deionized water (DI water) is used as a washing buffer to rinse the oil layer surrounding PEG hydrogel droplets. The DI water is poured several times onto polymerized PEG hydrogel droplets in the strainer then the droplets are picked up by pipet and re-suspended into water based solution.

From the observation of the washed droplet in figure 2.9, it looks much bigger than un-polymerized PEG hydrogel droplets. This is because hydrogel is the materials that absorbing water significantly. Its size is almost double.

The reason for having off-chip droplet gelation and washing process is because every step is easy to handle and there is no need to fabricate additional devices, such as droplet polymerization chamber and droplet washing chamber. However, this off-chip process has some critical problems. The majority of droplets are broken during washing step because

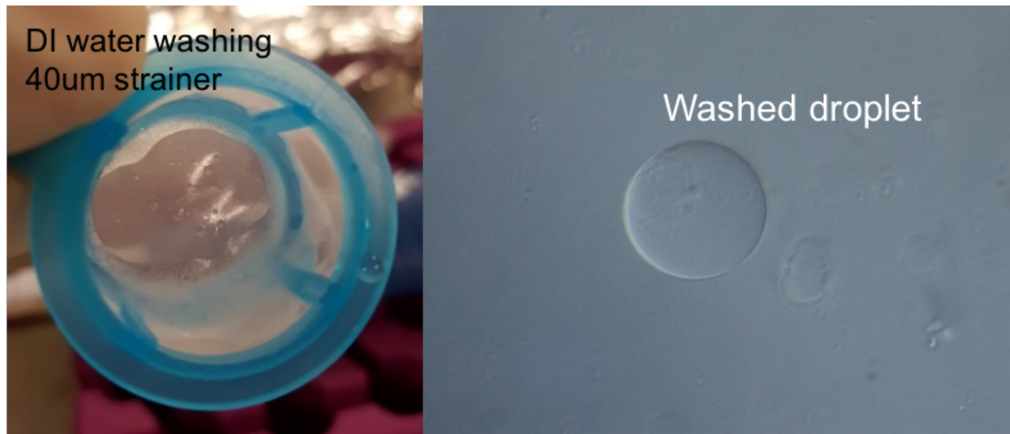

Figure 2.9: Washed individual PEG hydrogel droplet

of tough handling of pipet. Also, there are many droplet aggregations from figure 2.10 in water based solution after washing step. Consequently, it has low washing efficiency, thus, we could see only few droplets.

2.4 On-chip gelation and washing of PEG hydrogel

Due to the low washing efficiency from off-chip droplet gelation and washing process, it is inevitable to have the on-chip process to avoid tough handling of pipet and minimize the pressure to droplets. Droplets can be generated from the same T-junction from the off-chip process. Then, they will be collected in micro tube for few hours. For the fine polymerization during droplet's traveling on chip, the traveling distance should be calculated based on the UV exposure time. The total flow rate in the polymerization chamber is 380ul/hour and the UV light exposure range is 1.5cm. By calculating the flow rate and UV exposure time, we can obtain the required distance and it can be included in the effective exposure range, which is 1.3cm. The total traveling distance is 879,600um and it is

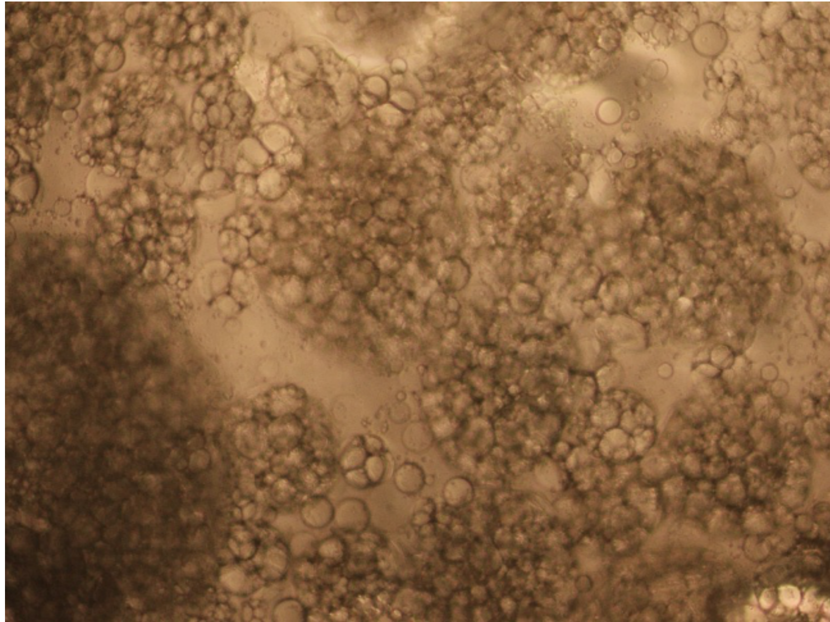

Figure 2.10: Droplet aggregation

meandered. In order to avoid the droplet merging issue in the meandered channel, which eventually cause non-uniform size of droplet and worse performance at cell culture, the oil spacer has been employed at the vertical channel from figure 2.11. The UV light intensity is 12mW/cm2, which is higher than the off-chip process since the PDMS microfluidic chip can scatter and reflect the light. In figure 2.12, after on-chip polymerization, all the cured droplets will be collected to micro tube.

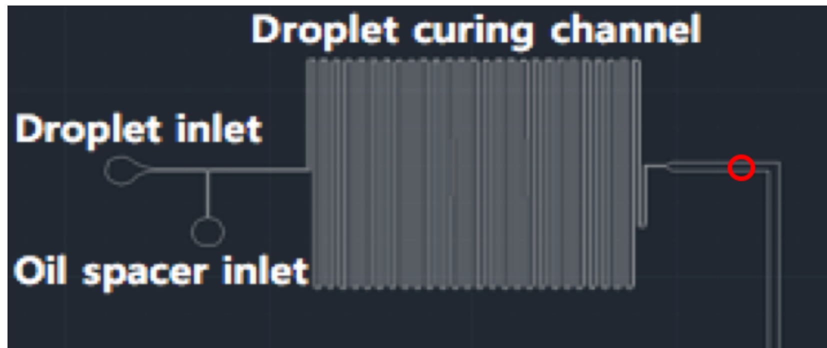

Figure 2.11: On-chip droplet polymerization channel

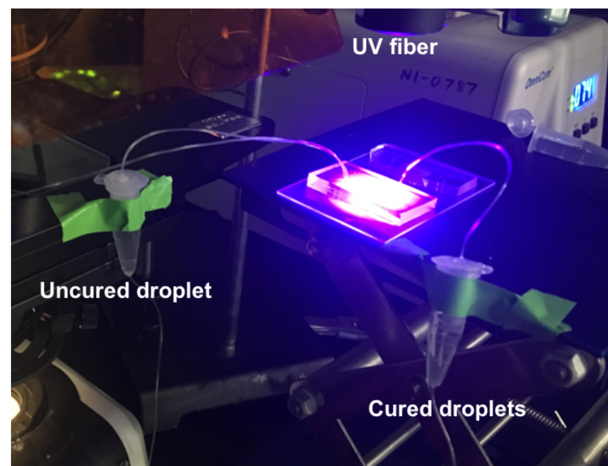

Figure 2.12: On-chip droplet polymerization setup

As it can be seen in figure 2.13, the collected polymerized hydrogel droplets are introduced into the washing chamber that having pillar structure. This structure prevents the droplet leakage since the gap between pillars is only 25um. The chamber has 3 inlets for water and droplet introduction and 2 outlets for droplet transfer and releasing oil. When the droplets are introduced and packed inside the chamber, water will be flown from the side inlets so that water push all the droplets. Since the middle outlet is closed, droplets

cannot flow outside and only oil will be released through the oil outlet. All droplets are squeezed by adjacent droplets and water.

Once almost of oil is released, middle outlet will be opened and droplets will be transferred into secondary droplet washing chamber. At the secondary droplet washing chamber, washed individual droplets and its manipulation were expected if water phase can push all the droplets. However, even under high flow rate, for example 1000ul/hour, droplets couldn't be moved and transferred since a big group of PEG hydrogel droplets were stuck on the bottom of the chamber. Due to the sticky material property from figure 2.14, all the transferred droplets were combined as a group of hydrogel droplet and stuck.

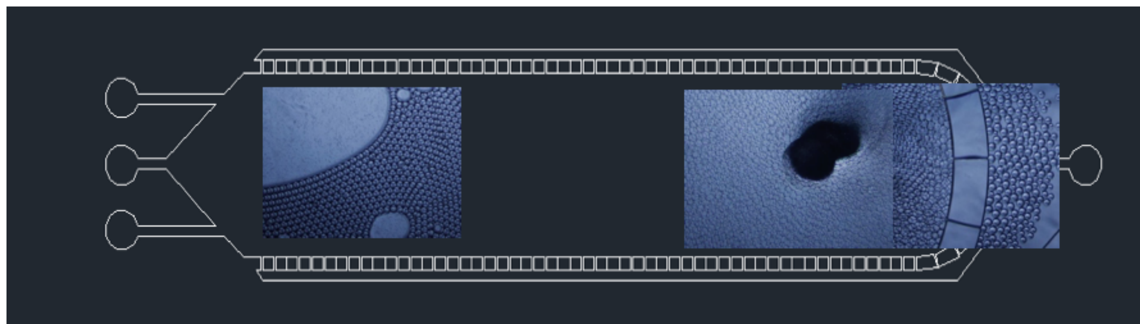

Figure 2.13: Droplet washing chamber

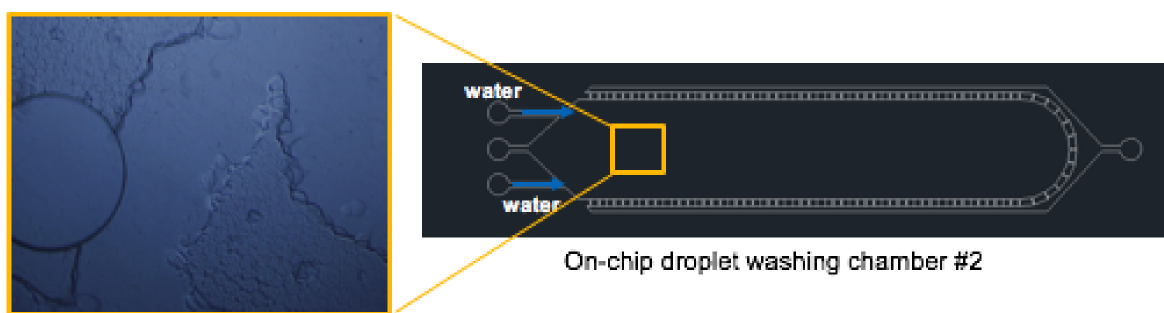

Figure 2.14: Secondary droplet washing chamber

The sticking problem is the biggest issue in using PEG hydrogel material. In order to solve the problem, we have tried several methods.

Two washing processes are conducted as a group washing process and individual washing process. The figure 2.15 illustrates the group washing. In the group washing, like the previous washing test, droplets were stuck on the bottom of the chamber and droplets couldn't be moved or transferred to make gel slab. In the individual washing process from figure 2.16, placing several branch channels can be a one way. The branch channel make droplets experience washing buffer so that oil layer can be washed. Putting electrode can be an alternative way to destabilize the oil layer. However, there was high resistance due to narrow channel width of branch channel. Thus, droplets couldn't travel and experience sufficient washing buffer. Another way of individual washing process is exchanging main stream from oil phase to water phase. Some people used pillar structure as a guide line for droplets to rinse the surfactant [17]. The pillar structure can be placed diagonal between two main stream chamber. In this case, the gap between pillars should be smaller

than droplet size. This technique can be expected to guide the polymerized PEG hydrogel droplets into water phase because water based liquid, which means without surfactant, can destabilize oil surface tension. Applying acoustic force can be a good method to push droplets artificially. The resonance frequency creates pressure node in the chamber. Then, all the polymerized PEG hydrogel droplets would be moved along the pressure node in water phase.

However, those methods were not successful since handling two liquid phase was not easy and also maintain laminar flow for both stream was challenge. Therefore, the polymerized PEG hydrogel droplets could not be transferred to another liquid phase.

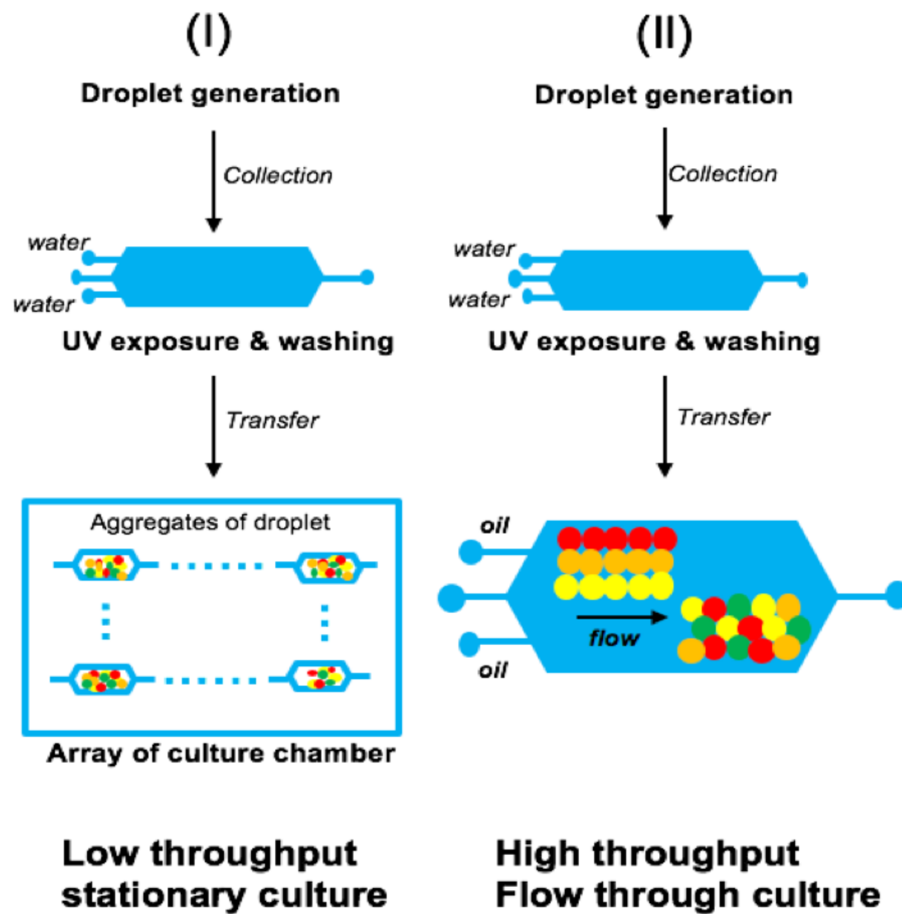

Figure 2.15: Group washing process

From the stick material property of PEG hydrogel material, it is mandatory to change the hydrogel material from PEG to alginate, which is classified as a natural polysaccharide material.

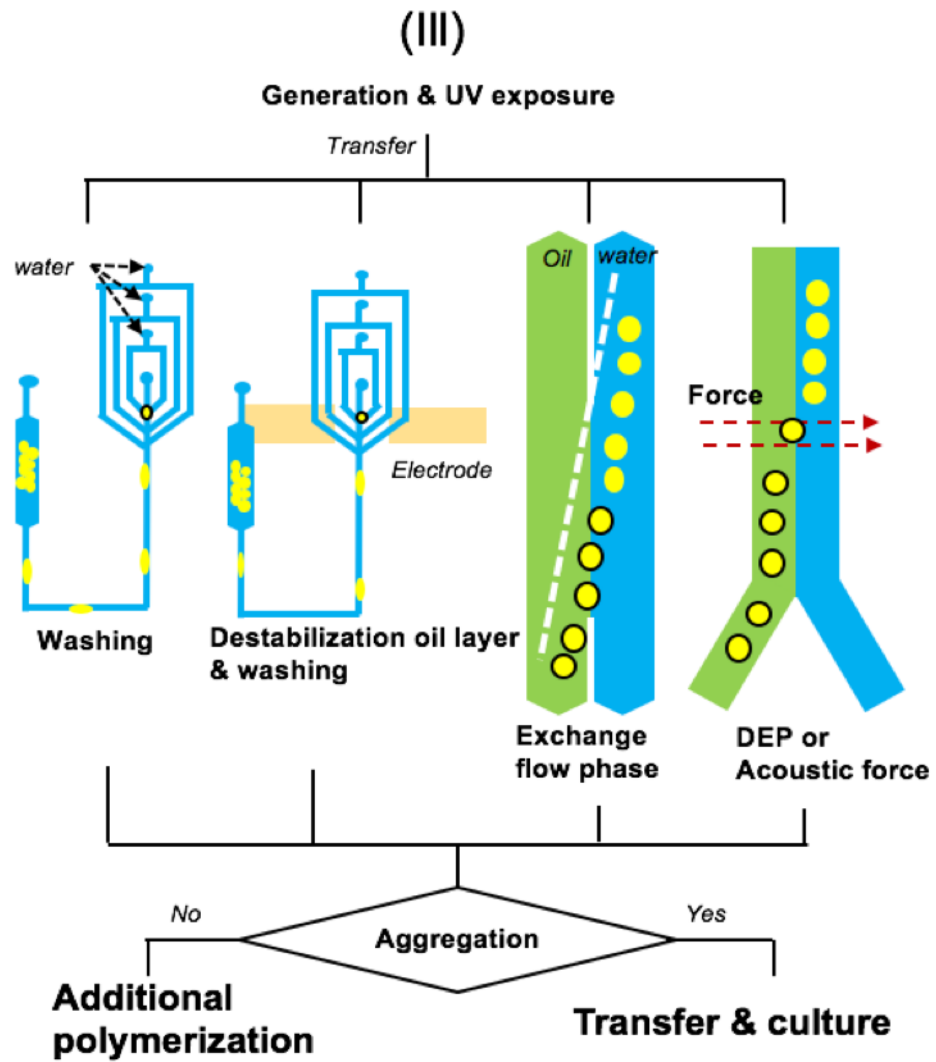

Figure 2.16: Individual washing process

3. CALCIUM-ALGINATE DROPLETS AS A SUITABLE FORM OF GEL DROPLET MICROFLUIDICS

3.1 Device fabrication and working principle

In this section, a droplet microfluidic chip based droplet generator is designed and fabricated. This microfluidic platform allows for studying the effect of flow rate on droplet size and cell encapsulation. In this design, a typical T-junction droplet generator is used and downstream is connected to allow accommodate huge number of droplets.

3.1.1 Soft lithography

The PDMS is a polymer widely used in microfluidics to make devices such as lab on chip. The PDMS is a transparent rubber-like material that can be obtained precise silicon based microfabrication, which is soft lithography.

The photo resist (SU-8) is poured onto 3” silicon wafer and spun with specific recipe. Then, the coated silicon wafer is baked on hot plate at high temperature. The first bake is called soft bake. Its aim is to evaporate the solvent to make the SU-8 photoresist more solid. This evaporation will make the photo resist layer to be changed and prepared for the UV exposure. The purpose of the UV exposure is to cross link the SU-8 photo resist by the activation of PAC (Photo Active Component). The PAC will change the partial property of the SU-8 which after baking will be soluble. To have successful exposure and fine

resolution, the photo mask has to be placed as close as possible to the SU-8 photo resist without any interfering. The existence of dust between the photo mask and the wafer will affect the perfect contact. This can be easily solved by the cleaning of the photo mask and precise manipulation during the UV exposure. After the UV lithography, the patterned silicon wafer is baked again. It is called PEB (Post Exposure Baking). After PEB, the photo resist is developed in SU-8 developer. At this step, the patterned design can be revealed on the substrate. Lastly, the silicon wafer is cleaned up by nitrogen blowing. This entire molding step allows the mass production of microfluidic chip.

As the last step of the soft lithography, the mixture of PDMS base and curing agent is poured onto silicon mold. Then, the mixture is degassed in vacuum chamber and baked at high temperature. The cross-linked mixture is taken off and finally the microchannel can be obtained in PDMS block.

3.1.2 Droplet generator and droplet reflowing chamber

The droplet generator and droplet reflowing chamber has been fabricated by soft lithography. The droplet generator consists of horizontal channel and perpendicular channel. The horizontal channel is for the continuous phase, which is mineral oil with 5% of span 80 surfactants while the perpendicular channel is for dispersed phase, which is 1.5% sodium alginate. In the fugure 3.1, the downstream is for the uniform droplets flow.

The droplet reflowing chamber, in figure 3.2, has pillar structure, which is for preventing the droplet leakage. Once the washed droplets are introduced into the reflowing

chamber, droplets can be packed and form a 2D slab of gel droplets.

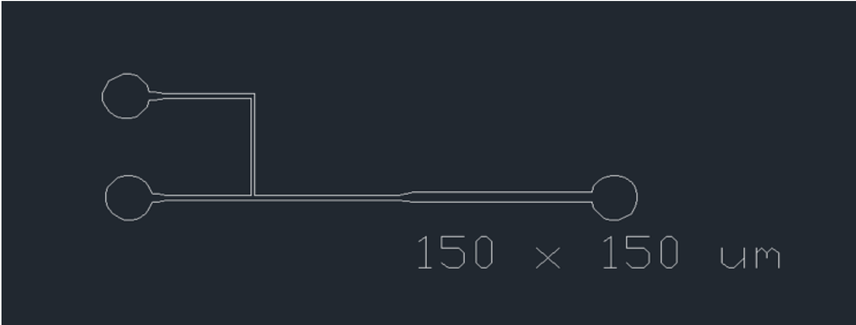

Figure 3.1: Alginate droplet generator

Figure 3.2: Droplet reflowing chamber

3.2 Material preparation

Among various hydrogel materials, a natural polysaccharide is the most frequently used material due to its biocompatible and capable of gelation under mild physiological condition. One of the most common material is alginate. The 1.5 wt % sodium alginate powder is diluted in DI water. In order to get the gelation, calcium chloride is used. The 0.1M of calcium chloride powder is also diluted in DI water. The degree of gelation can be controlled by the concentration of calcium chloride. However, the gelation is so quick, it is hard to get stable and firm gel despite of the sufficient calcium chloride concentration.

3.3 Result and discussion

3.3.1 Droplet generation

The 1.5 % of powder type sodium alginate is diluted in DI water and absorbed by 1mL plastic syringe (BD syringe) and transparent tubing is connected with the needle tip, connected with syringe. The mineral oil with 5% of span 80 surfactants is also absorbed by syringe and the syringe is connected to inlet of PDMS chip by tubing. By precise control of the syringe pump, either flow rate of both solution and droplet size can be changed. The generated alginate droplets travel from the outlet of PDMS chip to vial containing 400uL of 0.1M calcium chloride solution. The figure 3.3 shows the algiante droplet generation.

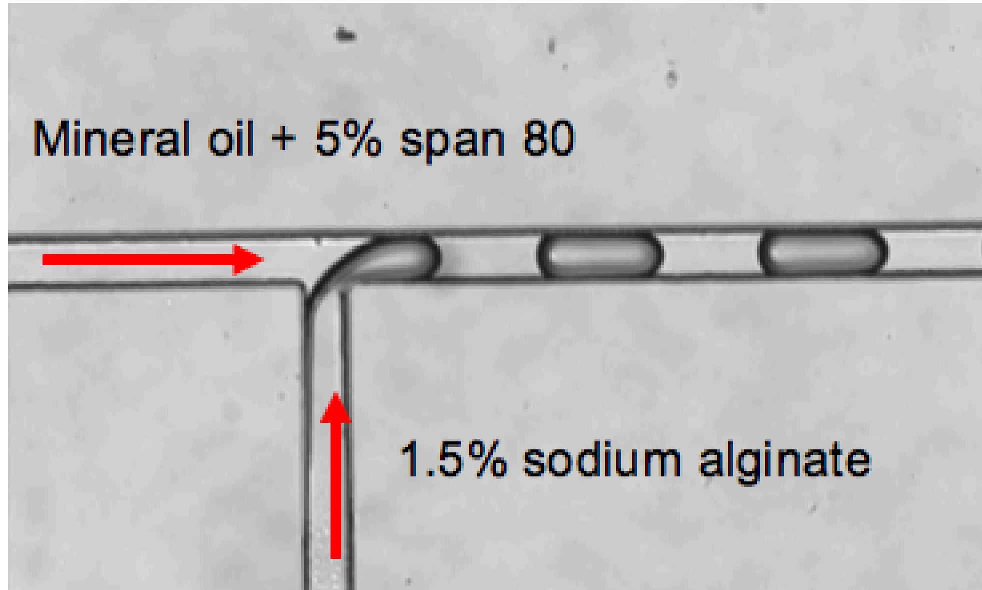

Figure 3.3: Alginate droplet generation

3.3.2 Gelation of Ca-alginate hydrogel droplet

In order to create the calcium-alginate hydrogel droplet, the dripping method has been used. The combination of calcium chloride and sodium alginate offers simple gelation method and it is less toxic than PEG hydrogels. Once the generated alginate droplets came out through the tubing, droplets meet the calcium chloride solution. At this point, the calcium can be diffused by water exchange into alginate droplets. Then, the ionic cross-linking can be happened by ion diffusion. This gelation is very simple and easy process. There is no need to have additional initiator, such as photo-initiator.

3.3.3 Droplet washing and manipulation

After the gelation of calcium solution and alginate droplets, droplets were collected for 3hours. Then, all the gelled droplets were transferred into centrifugal tube. All the droplets undergo centrifugation for 10min at 4400 rpm to separate all components. The majority of oil phase was separated after centrifugation due to its low density. Then, the floating oil layer on the top was removed by pipet. The calcium chloride solution was also removed. Lastly, the DI water was added and centrifuged again with same condition. At this last step, because of the lack of surfactants, the oil layer can be destabilized and separated from droplet surface. After that, all the droplets inside the tube were re-suspended into water based solution.

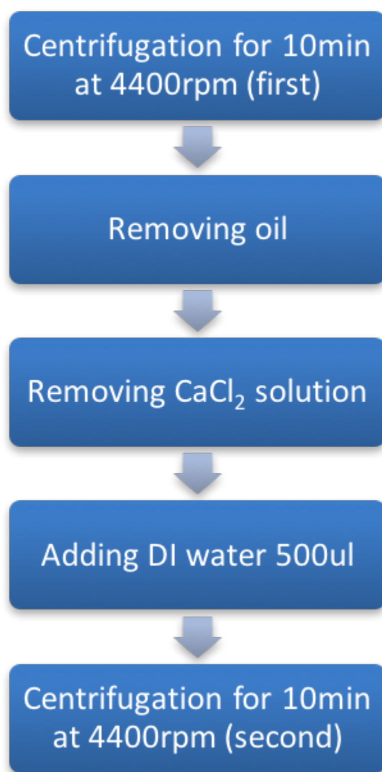

Figure 3.4: Droplet washing protocol

Comparaing to PEG hydrogel droplet washing process, calcium-alginate hydrogel droplet washing process yields much more washed individual gel droplets. The figure 3.4 shows the droplet washing protocol of calcium and algiante droplets and figure 3.5 shows the oill separation and washed individual ca-alginate droplet.

All the washed droplets were re-injected into reflowing chamber to manipulat them as a gel slab for the ultimate goal. By pushing water from the bottom of the micro tube, droplets can be re-injected. However, the washed individual droplets should experience high resistance and merging issue inside the tubing. Also, many droplets had been experienced high pressure during centrifugation. Therefore, only few number of washed

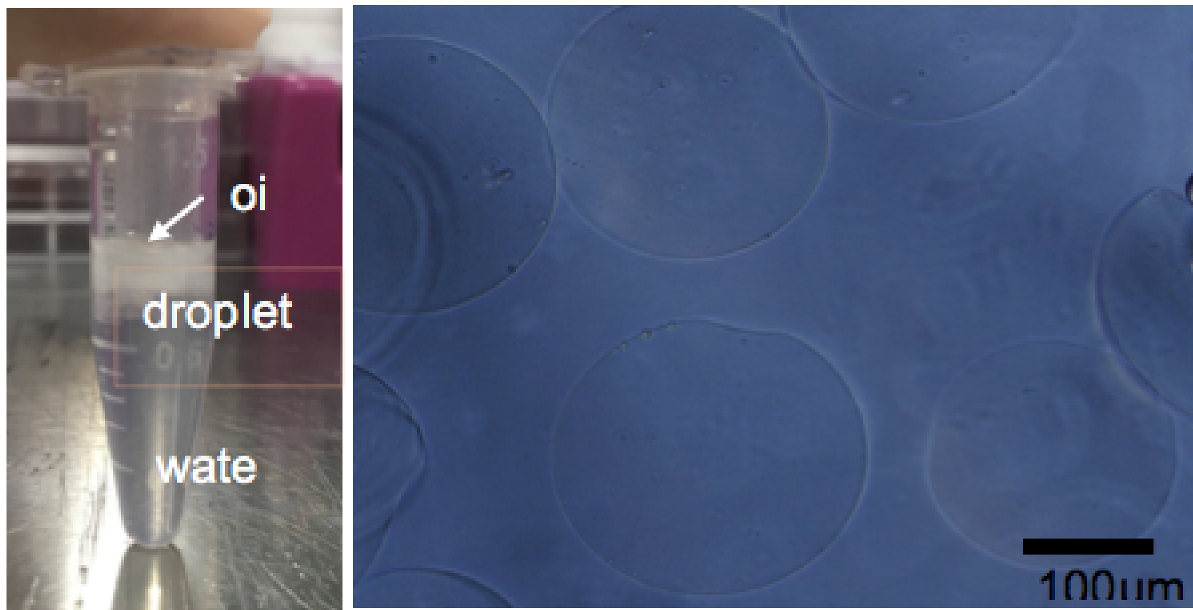

Figure 3.5: Oil seperation and washed individual ca-alginate droplet

calcium-alginate hydrogel droplets could be re-injected into chamber. One more possible reason for this issue can be an weak mechanical property of calcium-alginate hydrogel droplets. Among various hydrogel droplet, the calcium-algiante hydrogel droplets have the lowest stiffness. Thus, it can be broken either during centrifugation and during transportaion through the tubing.

From the result of reflowing droplets, the optimazation for stiffer calcium-alginate droplets should be required to avoid this problem. Changing concentration of calcium chloride or gelation technique can be an alternative.

In the obserbation of calcium-alginate hydrogel behavior from the figure 3.6, it looks not sticky on the bottom, which means this is way better situation than PEG hydrogel droplet case.

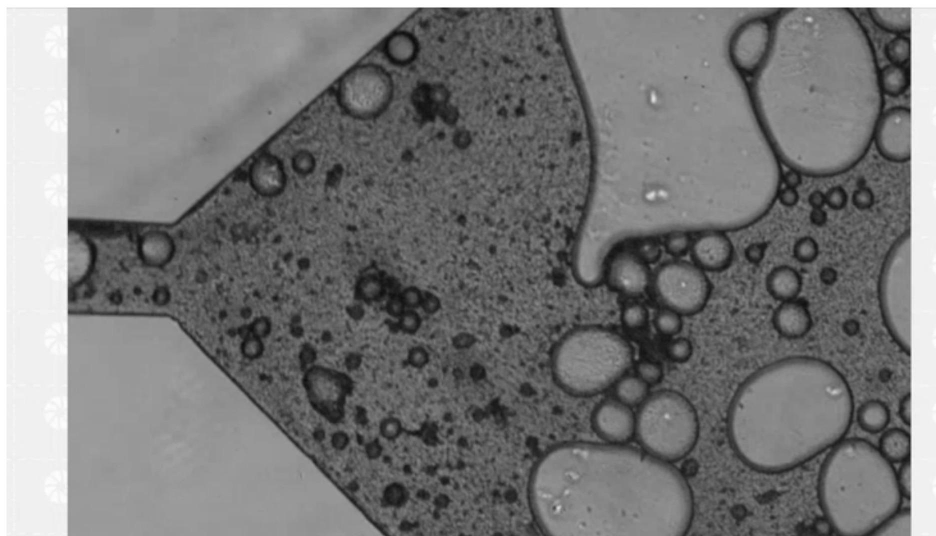

Figure 3.6: Re-injected ca-alginate hydrogel droplets

3.3.4 Cell encapsulation

The ultimate goal of this research is microbial screening at the novel platform. From this respect, the cell encapsulation in alginate droplet has been performed. The E-coli is used as a eukaryotic cell and TSB with 100ug/mL Kanamycin is used as a culture medium. The cell concentration is 4.776 x 106 CFU/ml. Target number of cell inside a single alginate droplet is about 10 cells. The cell encapsulation has been conducted at the typical T-junction droplet generator. For the encapsulation, the cells were mixed with alginate solution as an inner phase. After generation of cell-laden alginate droplet, all the droplets undergo gelation with calcium chloride in vial. Then, droplet washing protocol was followed. In order to see the cells in a single alginate droplet, the florescent detectable microscope was used. In the bright field image, the cells were located inside the alginate droplets. The droplet washing protocol also result in a nice washing performance.

After 24hours, droplets were stained by live/dead backlight bacterial viability kits (L7007) because the E-coli what we used for this test has no GFP expression. From the microscopic image in figure 3.7, the cells could be found showing different light intensity at SYTO filter and PI filter, respectively.

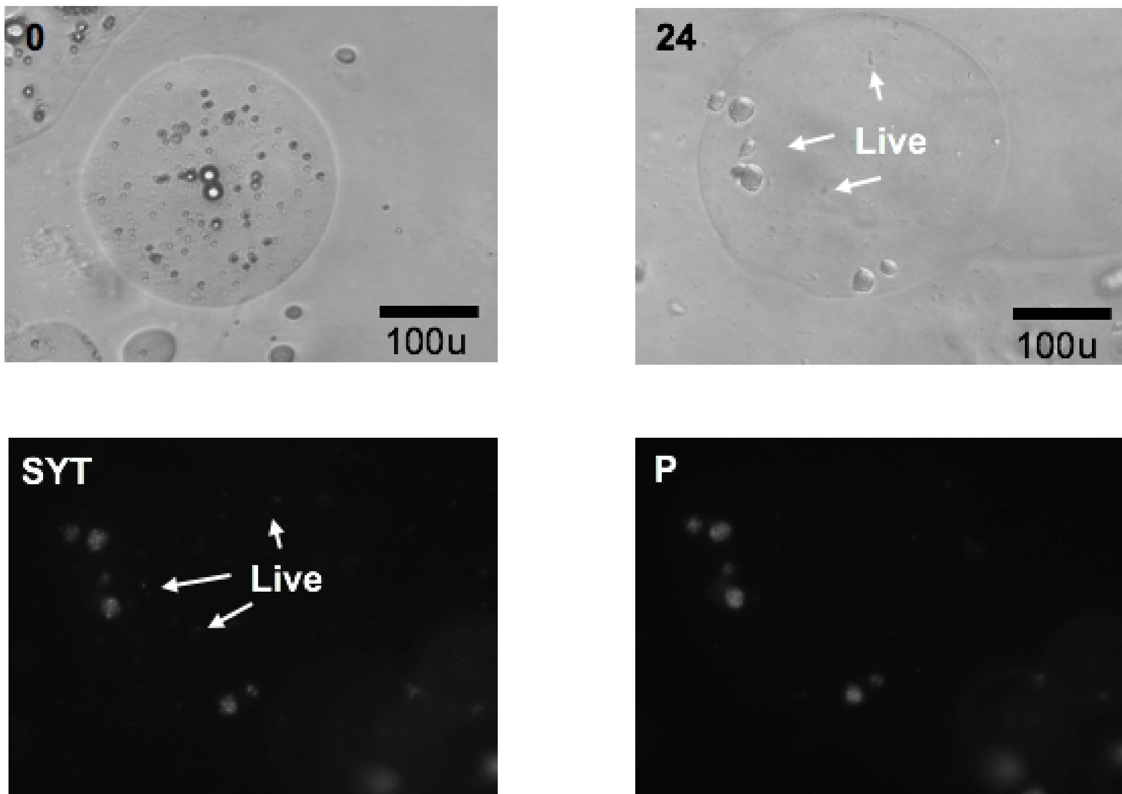

Figure 3.7: Cell encapsulation and its viability test

4. SUMMARY AND FUTURE WORK

4.1 Research summary

A comprehensive hydrogel study is conducted for understanding the characteristics of hydrogels. Hydrogels are polymeric network chains that can be obtained from either naturally or artificially. Based on its base material, the gelation method is also different. Among them, thermal cross-linking, ionic cross-linking, and photo cross-linking are the most common gelation method. Due to hydrogel's gelation possibility, they can be formulated as a slab, micro-particle, nano-particle and film. So, many researchers have utilized hydrogels as a transportation vehicle for drug delivery or a template for wounded spot tissue regeneration. But the most frequently used application is cell encapsulation for screening.

A PEG hydrogel droplet has been studied as a good material for gel droplet community. PEG hydrogel offers a good mechanical property and low degradation rate and its gelation control is pretty simple. The PEG hydrogel droplet generation was conducted by using a typical T-junction droplet generator and droplets were polymerized at off/on chip process. However, there were aggregation issue and sticking issue, respectively. Thus, replacing hydrogel material was inevitable.

A combination of calcium chloride and sodium alginate was a good alternative. Its gelation is very simple and no need to have additional initiator. Even though only the off-chip process was conducted at this time, still we had high efficiency with washed individ-

ual hydrogel droplets. Yet, additional optimization should be required for better reflowing performance but it will be a minor.

Lastly, the cell encapsulation was successful and its viability is also confirmed by using staining dye. However, less number of cell in a single alginate droplet after 24hours or long term period can be an issue that we need to deal with.

4.2 Future work

The on-chip process for generating calcium-alginate hydrogel droplet will be conducted in a high throughput manner. In this case, the more precise gelation control will be required. This on-chip process yields more uniform sized hydrogel droplets and more firm and stiff droplets, which is good for making droplet community in a 3D manner.

The cell culture point of view, long term period cell culture will be done for example 3days or 4days maximum to have robust cell viability. Therefore, initial number of cells for encapsulation in a single droplet will be changed for more survival after long period. Changing cell culture medium will be an alternative way for better cell culture.

As a long-term perspective, we will have a 3-D droplet community assembly, at which result in more effective cell-to-cell communications and nutrient exchange between different types of cells.

REFERENCES

[1] S.-Y. Teh, R. Lin, L.-H. Hung, and A. P. Lee, “Droplet microfluidics,” *Lab on a Chip*, vol. 8, no. 2, pp. 198–220, 2008.

[2] W.-L. Chou, P.-Y. Lee, C.-L. Yang, W.-Y. Huang, and Y.-S. Lin, “Recent advances in applications of droplet microfluidics,” *Micromachines*, vol. 6, no. 9, pp. 1249–1271, 2015.

[3] J. Wang, Y. Li, X. Wang, J. Wang, H. Tian, P. Zhao, Y. Tian, Y. Gu, L. Wang, and C. Wang, “Droplet microfluidics for the production of microparticles and nanoparticles,” *Micromachines*, vol. 8, no. 1, p. 22, 2017.

[4] S. Akbari, T. Pirbodaghi, R. D. Kamm, and P. T. Hammond, “A versatile microfluidic device for high throughput production of microparticles and cell microencapsulation,” *Lab on a Chip*, 2017.

[5] M. G. Simon and A. P. Lee, “Microfluidic droplet manipulations and their applications,” in *Microdroplet Technology*, pp. 23–50, Springer, 2012.

[6] T. Rossow, P. S. Lienemann, and D. J. Mooney, “Cell microencapsulation by droplet microfluidic templating,” *Macromolecular Chemistry and Physics*, vol. 218, no. 2, 2017.

[7] Y. Yu, R.-X. Wu, Y. Yin, and F.-M. Chen, “Directing immunomodulation using biomaterials for endogenous regeneration,” *Journal of Materials Chemistry B*, vol. 4,

no. 4, pp. 569–584, 2016.

[8] S. Mura, J. Nicolas, and P. Couvreur, "Stimuli-responsive nanocarriers for drug delivery," *Nature materials*, vol. 12, no. 11, pp. 991–1003, 2013.

[9] Z. Zhu and C. J. Yang, "Hydrogel droplet microfluidics for high-throughput single molecule/cell analysis," *Accounts of Chemical Research*, 2016.

[10] S. R. Caliari and J. A. Burdick, "A practical guide to hydrogels for cell culture," *Nature methods*, vol. 13, no. 5, pp. 405–414, 2016.

[11] L. Gasperini, J. F. Mano, and R. L. Reis, "Natural polymers for the microencapsulation of cells," *Journal of the Royal Society Interface*, vol. 11, no. 100, p. 20140817, 2014.

[12] T. R. Hoare and D. S. Kohane, "Hydrogels in drug delivery: progress and challenges," *Polymer*, vol. 49, no. 8, pp. 1993–2007, 2008.

[13] G. M. Whitesides, "The origins and the future of microfluidics," *Nature*, vol. 442, no. 7101, pp. 368–373, 2006.

[14] J. Atencia and D. J. Beebe, "Controlled microfluidic interfaces," *Nature*, vol. 437, no. 7059, pp. 648–655, 2005.

[15] H. A. Stone, A. D. Stroock, and A. Ajdari, "Engineering flows in small devices: microfluidics toward a lab-on-a-chip," *Annu. Rev. Fluid Mech.*, vol. 36, pp. 381–411, 2004.

[16] N. L. Jeon, H. Baskaran, S. K. Dertinger, G. M. Whitesides, L. Van De Water, and M. Toner, "Neutrophil chemotaxis in linear and complex gradients of interleukin-8 formed in a microfabricated device," *Nature biotechnology*, vol. 20, no. 8, pp. 826–830, 2002.

[17] H. S. Kim, A. R. Guzman, H. R. Thapa, T. P. Devarenne, and A. Han, "A droplet microfluidics platform for rapid microalgal growth and oil production analysis," *Biotechnology and bioengineering*, 2016.