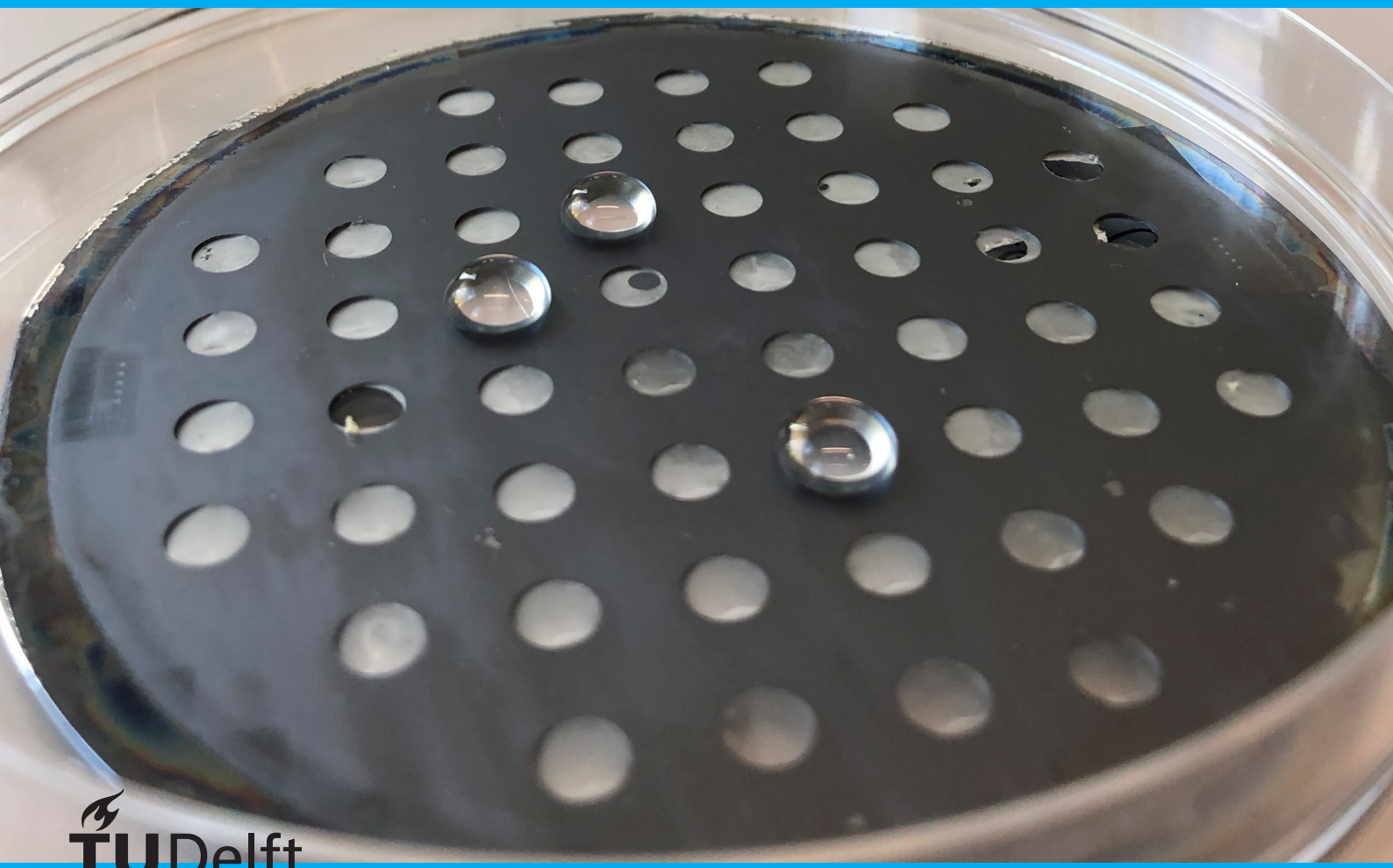
Fabrication of a flexible ECM supporting-membrane for organs-on-chip applications

M.I. Rosencwaig Lustig

October 24th 2019



TU Delft

Fabrication of a flexible ECM supporting-membrane for organs-on-chip applications

by

M.I. Rosencwaig Lustig

In partial fulfilment of the requirements for the degree of
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TU Delft

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M.I. Rosencwaig Lustig
Delft, October 2019

Preface

This project is part of the requirements for obtaining the degree of Master in Science in Biomedical Engineering. The thesis work took a lapse of 12 months, and by the end of it, the integration between an ECM supporting hydrogel and a customized OSTE-PDMS membrane was successful. The process started with a literature review to investigate what had been done in the past and to investigate further to the possibility of using a new material using a different formulation of PDMS. The process continued with building the flowcharts for the fabrication stage of the project. The second stage was the mechanical characterization of the OSTE-PDMS a polymer similar to regular PDMS. During this stage, collaborations between faculties were crucial. Viscoelastic testing was done with the help of Applied Sciences, and tensile testing was performed with the help of 3mE. The next phase was inside the cleanroom for the fabrication of a suspended porous membrane made of OSTE-PDMS. During this stage several tests had to be done since this was the first time the material was handled in the cleanroom and prior recipes or processes didn't exist. Once the suspended porous membrane fabrication was complete. The last part of the project was to test the bonding between the hydrogel and the polymer. With the help and collaboration of the University of Leiden, the bonding was tested and processed and by the end of the process, a successful device integration between the surfaces obtained.

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Delft, October 2019

Abstract

One of the most widely used materials in the Organ-on-Chip industry is Polydimethylsiloxane (PDMS). This material offers desirable features such as customized mechanical properties, patterning, biocompatibility, optical transparency, permeability to gas, viscoelasticity and hydrophobia. One example of Organ-on-Chip (OoC) is the Cytostretch platform proposed at TU Delft, where different tissues can be cultured in one single OoC platform. Its PDMS membrane can inflate and deflate in a cyclic and controlled matter, introducing mechanical cues to the cell substrate, and to the cell-cell interactions. 3D stimulation and custom patterning of the surface has shown to affect cells fate. This network provides both mechanical support and optimal cues for a more accurate cell formation. A new material appeared in literature as a substitute material for PDMS: off-stoichiometry-thiol-ene PDMS (OSTE-PDMS). It has similar characteristics as PDMS, but without known issues as in the absorption of molecules, swelling and gas leakage. OSTE-PDMS offers a modified surface to allow for specific functional groups to covalently bond to it. The physical bond between the layers ensure the transfer of the stimuli produced by the device, directly into the cell culture inside the hydrogel. Hydrogels are generally used in the cell culturing industry as these offer a 3D interface for biochemical and mechanical cues, as well as an optimal environment for cells to survive. The goal of this project is to integrate an Extra Celullar Matrix (ECM) supporting platform to a porous membrane made of OSTE-PDMS ensuring local adherence between layers. OSTE-PDMS was used to obtain a thin microporous membrane and its surface was modified to bond with thiol reactive hydrogels. Samples of PDMS, OSTE-PDMS, OSTE-PDMS + PEG DT (UV Cured Hydrogel) and OSTE-PDMS + PEG ODT (Chemically Cured Hydrogel) underwent tensile tests which were analysed and compared to literature showing results to be not similar in value but of the same behaviour. UV cured and chemically induced hydrogels were tested to physically bond with the modified OSTE-PDMS surface in a petri dish. Both types of hydrogels proved to be possible candidates as both successfully bonded. During the project the chemically induced hydrogel was preferred due the its success in bonding to an OSTE-PDMS patterned and suspended membrane. This hydrogel has better mechanical properties, is not UV dependant and has a quicker curation time, making it a great option for future projects.

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1 Introduction

Drug developing is a long costly procedure with an estimated cost of up to 1 billion US dollars. In the past 20 years the amount of new drugs released to the market has shown to be less and less[5, 12, 46]. This can be explained partially because several drugs had failure rates, which required them to be called back for newly found issues that were not detected in the clinical trials. [63] Lots of money is being invested by the pharmaceutical industry to introduce new products[5, 20]. Continuous innovations are made because of the rise of new methods to read measurements that otherwise would be impossible to obtain [22]. New microfluidic devices are being used to experiment on cell biology; cells are being cultured and inserted *in vitro* in a device which is designed to resemble *in vivo* mechanics.

Conventional static cell cultures and animal models are limited in mimicking the human cellular environment *in vivo*. 2D cultures exhibit a different biological function than 3D cultures, differences in enzyme activities, morphology, and metabolism of drugs. Cardiomyocytes have failed to develop functions in 2D cultures [27]. In contrast, 3D environments provide the cell the space to be able to interact with the extracellular complex and dynamic biomolecules [11] and mechanical cues from the substrate and to establish connections between its neighbours allowing it to have better proliferation opportunities.[3, 36]

Organ-on-Chip might be the solution to the current dilemma in the pharmaceutical industry. These are a microfluidic culture devices, designed to provide different tissues such as lung [15], intestines [27], heart [40], and the blood-brain-barrier membrane [4]; the ultimate goal being Human on Chip [41]. The device mimics crucial attributes of physiological conditions of what a functional living unit of the organ requires to function, which can be customized as required to simulate the tissue microenvironment.

Evidence has shown that cells that were in a 2D culture have different nuclear shapes, expression of genes and response to proteins. It is known that 3D culturing helps to improve the cellular differentiation and function as it provides a better platform in vitro to simulate the *in vivo* environment experienced by the cells [36], as cells depend on the microenvironment to functionalize [3] and improve levels of tissue organization [27]. 3D culturing has shown to have better surfaces than 2D cultures as it provides a greater surface area, it has been shown to metabolize drugs similar to *in vivo* cells [3]. 3D culturing is in part achieved by hydrogels, these materials are flexible, biodegradable and biocompatible. They provide the 3D network to the cell, mechanical support and optimal cues for the cell's differentiation.[13, 34, 36], it also enhances tissue organization and better expression of genes. [27] A good cell-cell interface and a good cell-substrate platform are key factors to promote a good functionalization of the cell cultures. Cell-ECM interface promotes cell growth, differentiation, proliferation, migration, and matrix remodelling. [9–11]

The Cytostretch platform [19] can provide a mechanical cyclic stress to a membrane made of PDMS. This membrane can be actuated pneumatically to transfer the pressure to the membranes. This design compared to other OoCs has the benefit that it can be customized to have a specific patterning in the membrane and the ability to integrate electronics for *in situ* measurements. Although this device has shown a lot of potentials, the surface of the material needs to be modified so that ECM supporting hydrogels can attach. A good integration between the interfaces of the hydrogel and the OSTE-PDMS will ensure the transfer of the stimuli from the device to OSTE-PDMS membrane and finally to the cell culture inside the hydrogel. In this project, we use a novel material OSTE-PDMS. This material shares similar characteristics to PDMS, but also provides a specific modified surface which gives focal points for a hydrogel to structurally bond. This method is based on previous results by [19, 38, 48] where a similar device was fabricated.

1.1. Problem Statement

As stated previously, the processing of OSTE-PDMS is new, the steps of building the proper fabrication process require the creation of new recipes, new measurements and every tool to be fine-tuned to the polymer characteristics; several trial and error samples had to be processed to achieve the correct values. Therefore, the fundamental research question of this thesis is:

"Can an OSTE-PDMS porous membrane be fabricated using similar cleanroom process flows as in standard PDMS?"

One of the reasons OSTE-PDMS is used during this project, is because this polymer formulation is able to provide a modified surface for the hydrogel to be able to attach to the surface of the membrane. Researchers have previously worked with the materials used during this project; none of them have provided a custom patterned surface. Hence, a second research question is:

"How can one provide a custom patterned OSTE-PDMS modified surface for an ECM supporting material?"

Once the material is processed, the performance and mechanical properties are measured using tensile testing curves to calculate its elasticity and viscoelasticity. Once the first stage is complete, the fabrication of a suspended OSTE-PDMS porous membrane device is created inside the cleanroom. Finally, the last stage of the project is to test if the hydrogel is able to bond to the porous platform.

1.2. Research Goals

This report is divided into individual research goals. Each research goal has been assigned a separate chapter.

1. The introduction where the motivations, problems and goals are explained. This helps answer the 'Why'

2. A literature review to allow me find a proper material that satisfies my research questions. This helps answer the 'what' has been done and 'where to go'

3. Design and Fabrication of the Porous Membrane. It presents the mechanical properties and the work done to achieve the final design. Its focus is in the fabrication process. It helps to answer the 'How'

4. Integration with the hydrogel. This section explains the steps followed for the creation and bonding of the materials. It helps to answer the 'How'

5. Conclusions on the project. This chapter provides analysis and evaluation of the exposed results in the project. And provide recommendations and future work to be done.

1.3. Thesis Outlook

The process of this work is based on the approach developed by [19, 39, 48] the fabrication of a custom porous membrane. Additional aspects studied in this project all depended on the success of the membrane. In the next section, a small summary of each chapter is provided.

- Chapter 1 - Introduction: This chapter captures the motivation, current problems and direction of the project

- Chapter 2 - Literature Overview: background literature was performed, allowing the research questions to be answered. Attention was given to biocompatible properties of PDMS, mechanical characterization of PDMS, use of PDMS inside cleanroom facilities and its capability to integrate with electronics and other materials. ECM supporting materials are key in the cell culturing industry.

- Chapter 3 – Design and Fabrication: This chapter exposes all the fabrication processes of the suspended OSTE-PDMS porous membrane. Design stages of project show all the tests performed on the OSTE-PDMS, tensile, and dynamic tests were done to obtain elastic and viscoelastic information to help us characterize the material. Also, during the fabrication stage several tests had to be done to optimize the process, new recipes and new processes on tools had to be created to obtain a working device.
- Chapter 4 – Hydrogel Bonding: The idea behind the project is to be able see if an ECM supporting hydrogel can integrate to the custom patterned OSTE-PDMS
- Chapter 5 - Conclusion - This chapter analyses the results and helps to bring answers to our problem and research goals. Recommendations and future work are also mentioned in this chapter

2

Literature Review

During this stage research articles and new published devices are studied for initial background investigation to understand what has been done and what are the next steps. In this section the topics are exposed from a general to specific order. Each topic of the literature review is intended to go from a general view of what the OoCs are and followed by a more specific topic to show the direction and goal of the project.

2.1. Organs-on-Chip

Organs-on-chip or OoC use conventional, cleanroom compatible semiconductor manufacturing process [19] to engineer microfluidic culture devices, that simulate the environment, form, and function of living human tissues, tissues such as the lung, skin, kidney, gut, cancer and blood-brain barrier (BBB), among others. [16]. The main design goal of OoC, is to mimic a living organ system, to emulate their essential functions as a whole [63].

When designing these chips, important factors need to be determined in able to define the level of complexity. Mechanical and functional characteristics, different parameters, and readouts need to be considered to be able to serve the intended purpose of the chip [51]. Such chips are designed to resemble more accurately the interactions between different cell types, tissues, and organs, in contrast to mimicking interactions between cells of the same type in 3D models. The simplest system to simulate these interactions is a perfused chamber containing the cultured cells and a single microfluidic channel. More complex designs are created using multiple microfluidic channels and utilizing a porous membrane to simulate the interface between tissue-tissue connections such as the blood-brain barrier. [2].

These microfluidics chips offer a huge opportunity as an alternative to traditional animal testing and clinical trials. They are a living, three-dimensional cross-section of units of organs, these units will recreate aspects of the human-specific behaviour in a controlled manner and may include fluids, strain, stretch, and airflow. [19] OoCs present a device to model human physiology *in vitro* that can research molecular, cellular scale activities that occur in organ functions such as tissue function and the signalling pathways to transform healthcare. Also, they can be used to recreate human-specific disease states, as well as the continuous look for new therapeutic targets *in vitro*. [2, 51]

OoCs are often fabricated with a process similar to the one in **Figure 2.1**. Microfabrication techniques are used, allowing wafer-scale processes and integration with silicon electronics. First, the silicon wafer will be spin-coated with a photoresist; on top of the photoresist the photomask will be used to provide the patterning. Ultraviolet light will be used to expose the photomask, the mask will protect the patterned parts on the silicon, and the UV will dissolve the other parts, obtaining a patterned photoresist. The liquid polymer will be placed on top of the photoresist to be polymerized and peeled off to obtain the polymer Stamp.

One example of a two-chamber,two inlets followed by a single-channel device with one outlet is in **Figure 2.2**; the Polymer stamp is placed on top of the silicon containing the desired pattern, then the stamp obtained is inverted, obtaining the pattern of the microfluidic channel in the polymer and the openings at both ends. Then the polymer is sealed into a smooth surface such as glass, which will create the microfluidic channels used by the cells to move. This central channel needs to be coated with ECM molecules and flowing cells so that they can set into the ECM, continuous perfusion with culture medium is required.

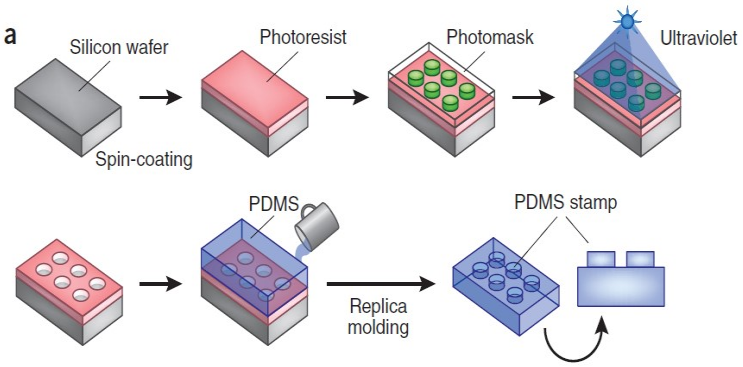

Figure 2.1: Example of a process to obtain a PDMS stamp.Taken from [2]

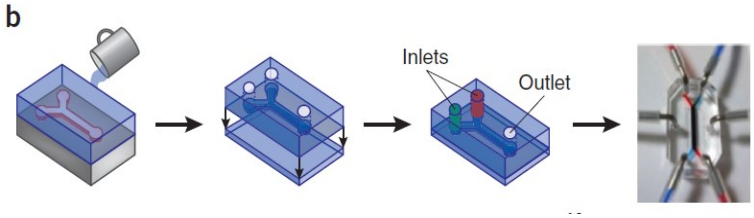

Figure 2.2: A single-channel microfluidic device using a PDMS slab for the inlets, outlet, and a microfluidic channel. Taken from [2]

The goal is to be able to integrate several different organ-specific chips and be able to recreate a human on a chip.[33] These chips would be connected through each other with microfluidic channels with a controlled flow distribution and also ratios allowing them to physiologically couple and create *in vitro* models of subsystems. [51].

One way to see the development of different types of OoCs is in **Figure 2.3**. In this example we can study the *in vivo* minimal functional units that we need. For a heart muscle (A-C) we require contractility and electrical activity, and we define our minimal functional unit as a strip of cardiac cells. Electromechanical stimulation is simulated to reproduce functionality. A BioWare is a strip of cardiac tissue inside a hydrogel that can be both electrically and mechanically stimulated. Readout data of the chip is the beat rate, contractility, and absorption rates.

Lung chips (D-F) require an air-liquid interface and lung absorption. The minimal functional unit is defined as a single alveolus, using a mechanical force that repeats itself cyclically successfully mimics the breathing ventilation process. The design will consist of two layers of epithelial and endothelial cells separated by a mechanically stretched porous membrane. Readout data such as cell imaging and dissolved gas concentration is extracted from the design.

Additionally, these devices can be designed to be able to be affected by 3D mechanical forces that provide stretch ability and compressibility to the environment, and this is applied to simulate the environment of the cells and the physical microenvironment of living organs allowing us to study mechanobiological responses of the cells.[2, 39, 61]

2.2. Cytostretch

The Cytostretch, a customized membrane platform proposed by Gaio in TU Delft, is a way of having an OoC capable of being tailored to fit different OoC applications. In contrast to the current focus of OoCs on a specific design of a specific functional unit. [19] This membrane can be customized to fit different needs such as porosity to allow signalling pathways between tissues in different compartments, a stretchable electrode array for in situ stimulation and data gathering, specific micropatterning to allow cell alignment and mechanical stimulus to generate stress on the substrate.

Most OoCs rely on cellular markers to identify different information in these models and leave out of the equation the 3D mechanical cues. These mechanobiological cues are very important in the design considerations as they are the standard function metric in systems such as heart, lung, gut, and cartilages. To address

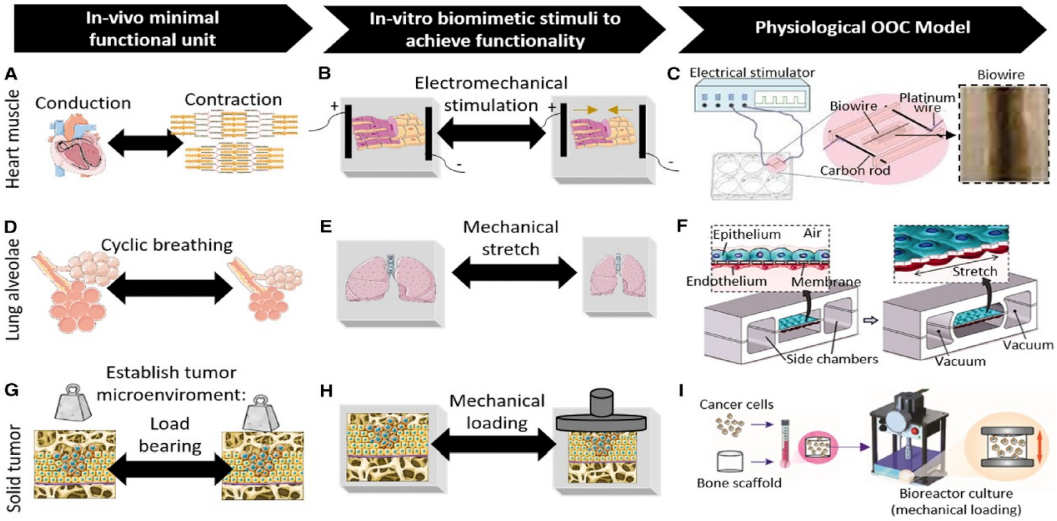

Figure 2.3: Considerations for Heart and Lung Devices. Taken from [51]

these needs, a mechanic stretchable design is used. [39]

The most important element of the Cytostretch membrane platform is the membrane. This part of the design consists of a free-standing membrane made of PDMS; this membrane will be shaped as a dog-bone and integrated into a silicon chip as shown in **Figure 2.4**.

The membrane is made out of PDMS, this material is used widely in OoC, and it has a few advantages such as customized stiffness and elasticity of the membrane, which allows it to be cyclically inflated and deflated using a pneumatic system. The design of the dog bone shaped membrane allows to provide not only vertical displacement but also lateral to the tissues, having the membrane inflated with a pressure of 40KPa you can get a displacement of 500 µm [19] additionally the membrane is cleanroom compatible making it not only compatible with the cells but also with silicon, being able to pattern directly on the membrane to obtain specific designs on the surface.

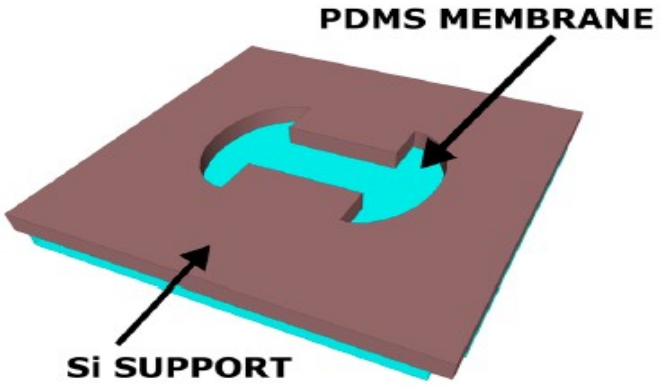

Figure 2.4: Back View of the Cytostretch membrane platform. Taken from [19]

Figure 2.5 provides a picture of the final chip where we can identify the electrode array available, the four cultivation chambers and the dog bone shaped PDMS membrane inside the chamber. The microfabrication techniques will allow integrating different features to the membrane without affecting the whole process speed. Allowing the platform to be used in different OoCs as required.

One example of a design like the Cytostretch membrane but using a different shape of a stretchable membrane can be shown in **Figure 2.6**. This design starts by showing the layering of the design; we can see where each layer and the expected behaviour of the stretchable membrane. Strain sensors are added to the membrane to give the readouts of the cell-seeded in the design; in part B the electrode is on top of the membrane

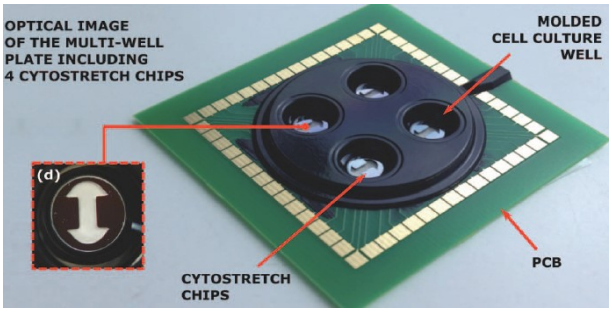

Figure 2.5: Example of the Cytostretch platform with electrodes proposed by [18]

for insulation. We can see the membrane stretching in C and D and finally in E we can see the device with the hydrogel and the cell culture placed on top. [39]

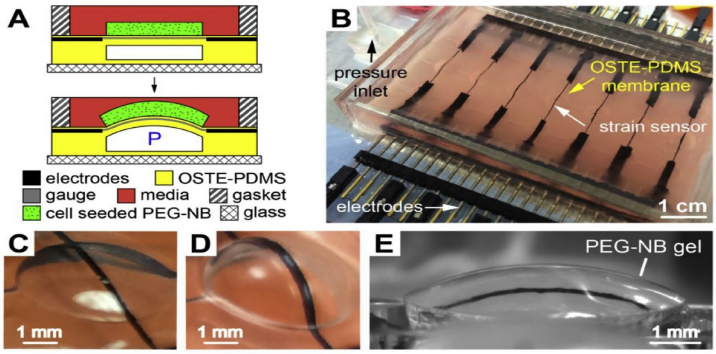

Figure 2.6: Stretchable membrane platform with electrode array to stimulate and to measure a cell culture. [39]

2.3. Extra Cellular Matrix (ECM)

The extracellular matrix is **Figure 2.7** composed of a 3D network surrounding the cells. The network consists of fibrous proteins (most prominently collagen), soluble molecules, and proteoglycans that create a 3D matrix providing support directly to the tissue and cells affecting its behaviour, therefore obtaining functionality. [21]

The features that the ECM is providing for the cells are stiffness, elasticity and mechanical cues such as topography. [61] Stiffness is a key factor in cellular morphology, migration, proliferation, cell markers, and differentiation to obtain a fully functional specific cell. [62]

The body has different kinds of tissues, the ECM surrounding those tissues needs to vary depending on the tissue. For instance brain tissue varies from 0.5 to 1 kPa and bone tissue varies from 10 to 20 kPa. This is something to look out for when deciding to investigate a specific cell and the environment which surrounds it. [61]

2D cultured cells and 3D cultured cells will exhibit different behaviour. ECM provides the mechanical and chemical cues facilitating cell to cell and cell to substrate behaviour. This is the reason why 3D cues are so important; there is no way to mimic the microenvironment in 2D [1, 36]. Studies show that cells cultured in a 2D environment show differences in shape, gene expression and the synthesis of proteins inside the cell. [35]

Here is an example of how important and flexible the ECM is in both communication and mechanical stimuli. When the body is injured and needs to produce a blood clot, the damaged tissue will release tissue factor, a protein receptor, that will adhere to another factor inside the ECM which will trigger the platelets to adhere to the walls and also stimulate the cells to contract and close the wound.

2.4. Hydrogel

In recent years development of new technologies have been designed to give the cultured cells what they require to acquire its morphology. Cultures that are laid over a rigid surface can proliferate but have poor cell

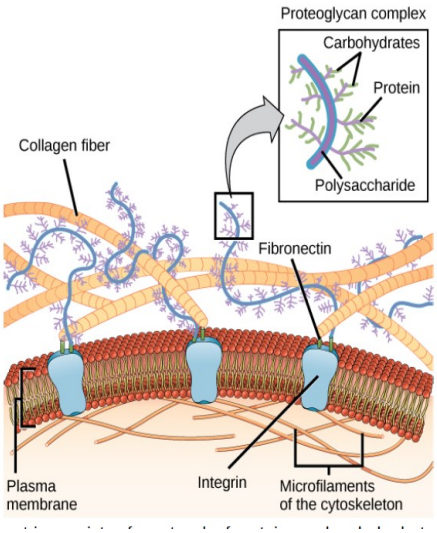

Figure 2.7: The extracellular matrix consists of a complex network of proteins and carbohydrates[43]

differentiation due to the lack of communication pathways [11]. In 3D cell cultures receptors and different molecules are more spread, whereas in 2D designs the binding proteins tend to concentrate on the surface [3].

The ECM microenvironment present multiple stimuli, not only with bio-functional and matrix molecules present for signalling purposes, but also simulating mechanical properties of the tissues [38]. These properties offer stability and the mechanic interface to transfer the stimuli to the cell. [7]. The mechanical properties of these materials are typically elastic modulus and shear modulus.

Various methods to simulate a 3D environment have been designed. Approaches can be very broadly categorized as scaffold and scaffold-free based, which can be made of natural or synthetic materials. A synthetic scaffold has the advantage as it can be customized in both its mechanical characteristics and chemical composition, key features that affect the differentiation of cells. To be able to offer an environment to proliferate, the synthetic material needs to be coated with ECM proteins to be able to mimic the ECM mechanics successfully. [35, 61].

Hydrogel systems are scaffold-based technology, they consist of polymeric materials and water, that are used to mimic the mechanical stimuli the ECM is providing to the tissue by making up the 3D network surrounding the cell [36]. Their formation involves the change of liquid precursors into solids by chemical crosslinking to organize the materials together.[7]. Mechanic characteristics such as stiffness, topography, elasticity and Young's Modulus all influence the cell. These properties vary from tissue to tissue due to the different tissue-specific interfaces [36, 61]. Additionally these microenvironments provide soluble and insoluble matrix space for the biochemical communication.[39].

The hydrogel is biodegradable and biocompatible with the body. Synthetic hydrogels are often made of polyethylene glycol (PEG). This material has good properties for use in OoC technologies as it has characteristics such as customized viscosity, and it can have porosity.[36] Different functional cues can be attached to the polymer via photo cross-linking with UV light.

These systems will encapsulate a cell culture inside a hydrogel, as seen in **Figure 2.8**. The cells are inside a matrix of proteins and molecules that are there to provide support for the cells as if they were in their natural environment, and additionally provides the signalling paths for them to communicate. Cell migration is a key factor in all tissue formation, and hydrogels are designed to provide that mean for the cells to move. [13]

Swelling is a mechanical property that has shown to influence the mechanical properties of the materials [30]. This property gives information about the hydrophilicity of the material and the crosslinking density. Evidence has shown that rigid substrates show less swelling in comparison to softer surfaces. [7] Additionally, different kinds of cells can be cultured together in the same 3D cultured hydrogel. The combination of using hydrogels, active agents and co-culture systems proves to work in providing a model for cell culturing.[35] These cells can be added into the hydrogel by self-assembly, cross-linking or polymerization by UV light. The UV light is a known factor of a bad effect on the cells, and also, the cells sometimes can be cultured for a small period due to problems with the transport of nutrients in the hydrogel. [35]

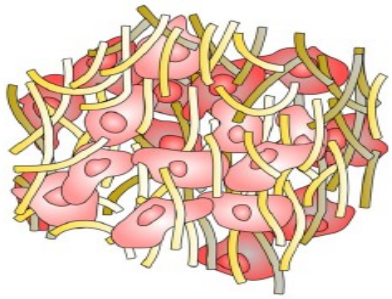

Figure 2.8: Cells mixed with a matrix of proteins using a water-based gel to provide additional chemical signals to the culture. [35]

These materials should be porous in the nanometer scale due to its role in survival, distribution, migration, interconnections, tissue regeneration, and healing [36]. This can be seen in **Figure 2.9**, where different microfabrication techniques produce different textures of the hydrogel. Porosity is correlated to swelling and mechanical properties of the hydrogel.

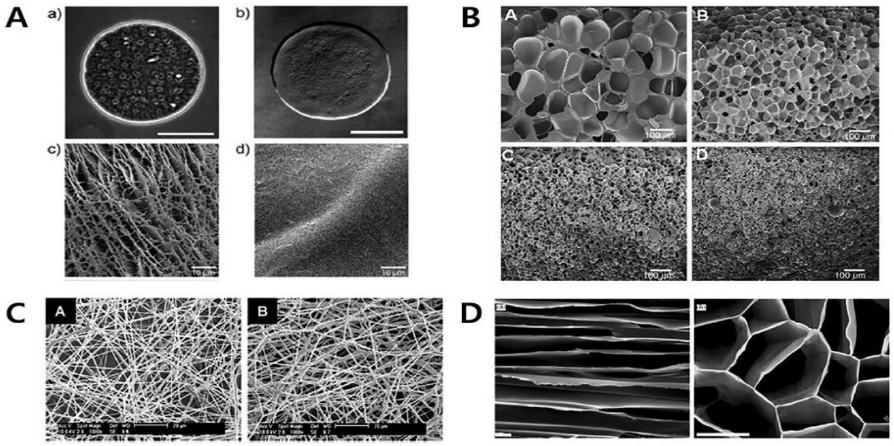

Figure 2.9: Different Techniques to control porosity. [36]

Degradation of the hydrogel over time has shown to affect its mechanical properties, absorb proteins resulting in swelling of the material, which will affect behaviours of the cells such as spreading traction. The hydrogel can sometimes desire these characteristics, and sometimes these are issues to be addressed. [7]. For example [39] showed that hydrogels initially degraded and softened, then by mechanical stimulation regained its stiffness, corresponding to the extensive cell network and collagen production.

Many studies show that microfluidic chips are very useful for the study of cell behaviour. Microfluidic chips are capable of simulating fluid concentration gradients in cell scale with a small quantity of cell culture and drugs. These chips are made of glass, plastic or silicone materials, which are different than the ones found naturally in the ECM; this makes them not very cell-friendly. Nevertheless, these materials offer a great interface for the cells as the surface is fully customized. The microfluidic paths are combined with hydrogels, which convert the channel to be biocompatible and help the cells to adhere to the surface of the lane successfully. [35, 38]

2.5. Polydimethylsiloxane (PDMS)

Polydimethylsiloxane (PDMS) possesses highly desirable characteristics, for that reason, it is a widely used material in biological applications. [47] and cell culture [57]. Several applications of PDMS-based microdevices are present in the literature, such as devices for cell culture and analysis, [16]

This material has key features that give it advantages over other materials. These characteristics are nontoxic, clean room and microfabrication techniques compatibility, allowing the material to be inexpensive, to have customized patterning, allowing it to be in high volume fabrication, biocompatible, optically transpar-

ent, permeable to gasses, impermeable to water and viscoelastic [19] [47, 55]. Additionally the stiffness is customized by altering the curing, modifying ratios of base agent, mixing different PDMS, adding molecules and using fillers, [59] obtaining substrates liquid or solid-like as required.[61]. Additional studies have shown the Young Modulus is also influenced by curing temperature and reaction time [61].

Nowadays, a lot of researchers use PDMS to seal into a 3D shaped structure by using soft lithography or replica moulding due to their easiness of process, transparency and a customized substrate. [57] These allow researchers to rapidly build prototypes according to their needs and test their applications. [19] Soft lithography helps us etch a pattern in chips (microfluidic lanes) made out of silicon. Also this method is more biocompatible and better with flexible materials [2].

Soft lithography is crucial in constructing multi-layer microfluidic devices that are interconnected. The use of multiple layers allows for each part to show a specific characteristic [54] Additionally to the mechanical properties, the protocols for soft lithography can be adjusted to achieve PDMS bonding to a substrate with an electronics layer to be able to measure different biochemical cues.

As shown in **Figure 2.10**, a porous PDMS membrane via microfabrication techniques, at first (a) the PDMS is mounted on top of the Silicon wafer coming out of the process flow PDMS is compatible with Silicone or other PDMS layers, next the aluminium is sputtered on top. The process continues by placing a layer of photoresist and then etched (c), step (d) allows us to see the pore array being patterned on top the PDMS, and a final etching will allow having the free-standing porous membrane. [19]

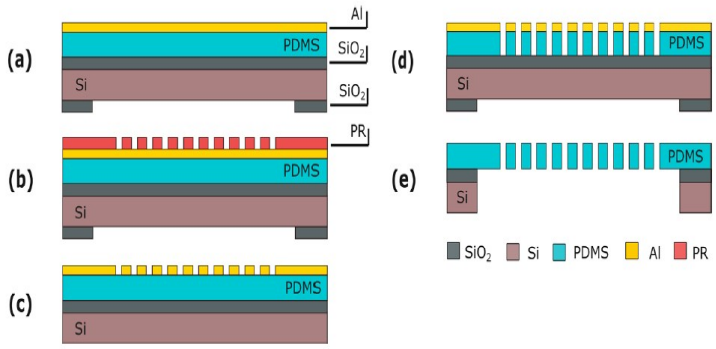

Figure 2.10: Micro-pore array for the membrane flow chart. [19]

Accessing these materials to design microfluidic chips give characteristics like biochemical compatibility, faster prototyping and lower production costs, the possibility of integrating electro sensing, and fitting optical needs. In the end, it will depend on the application requirements. [19, 59] Another example of a PDMS membrane used to provide mechanical cues to the tissue is in **Figure 2.4**. PDMS is widely used in OoC applications; the material can be used as a membrane and as a PDMS slab to provide to the model accurately the characteristics *in vitro* that the cells show *in vivo*. One example of a simple migration experiment is in **Figure 2.11**. Two PDMS slabs divided by a porous membrane, with a hole in the centre measuring 5mm, this creates two microfluidic chambers. The top chamber has cells seeded into it, while the bottom chamber is full of culture medium and proteins which attract the cells.

It is worth mentioning that additional experimentation should be made for adjusting tissue geometries and channel profiles, so that fluid residence times correspond to physiological residence times of body fluids in the targeted organs [19]

Although PDMS is a very good material, it has disadvantages such as hydrophobic surface characteristics and vapor porosity which affect the flow rate of the microfluidics. Additionally, the non-permanent surface gets non-specific binding of proteins, which affects the availability of target molecules. [6, 26] Evidence of swelling has been detected in PDMS and has seen to affect its mechanical properties [30]. The reason can be is its vapor porosity, gasses can escape leading to concentration changes, this affects the permeability of lipophilic molecules and affects the drugs study. PDMS due its tendency to swell its not compatible with some common chemicals. [24, 59] Synthetic polymers are inert; cells avoid this kind of surfaces making it impossible for them to proliferate and survive. [53] Evidence by different articles has shown that achieving good growth conditions will influence cell behaviour and function. [3]

Studies are taking into consideration the absorption of a small molecules in PDMS. Results have shown that the absorption is variable and time-dependent. Different theories explain that molecular weight and

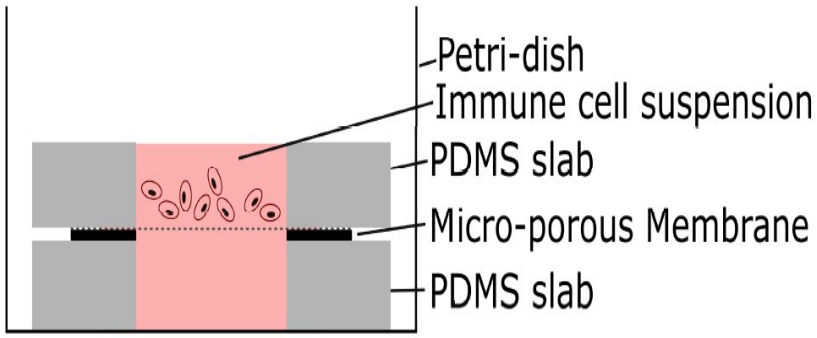

Figure 2.11: Simple setup of a migration experiment [19]

hydrophobia were determinant factors to the absorption of molecules in PDMS; however, studies need to go further to probe this theory successfully. As a consequence, the kinetics of drug absorption into the PDMS need to be accurately modelled, since part of the drug molecules will not reach their intended destination. Solutions to this issue suggest different coatings can be applied to the PDMS and improve the cell adhesion and block the absorption of molecules.

PDMS uses a high and long thermal energy to cure and change from a liquid state to a solid elastic state. One of its problems in large scale production is the individual requirement of surface treatment. To be able to bond molecules to the PDMS surface, methods such as plasma oxygen and silanization are used, but it has shown that it will reverse to its previous state in time and also can be incompatible with the fabrication [25] Additionally surface modification of the microchannels will not be possible using this kind of approach [64] Also glue can be used to attach different molecules to the substrate [23] but it will make the device more complex and harder to obtain

2.6. Off-Stoichiometry-thiol-ene Polydimethylsiloxane (OSTE-PDMS)

OSTE-PDMS is a type of polymer that is used to enhance cell adhesion by a passive treatment of the surface via a thiol-ene reaction. These formulations have an excess of one of the functional groups, hence off-stoichiometry to create a new polymer that has specific mechanic properties and carries very reactive functional groups in excess in its surface and bulk ready to bond. [8] If the formulation has an excess of thiol groups elastic and soft materials are obtained. In contrast an excess of allyl it will be very hard and stiff. [25] Usually these formulations are made with thiol-PDMS and vinyl-PDMS. [6] They offer the advantage of being capable of being very stiff or very soft depending of the ratios of the Thiols and Vinyl groups. [8]

OSTE-PDMS share some characteristics with regular PDMS. Characteristics such as elastic composition and optical transparency are still present, but the polymer now offers a passive one step permanent surface modification from where different molecules like PEG can be used. This material is capable of addressing issues with PDMS such as a greater solvent compatibility, antibacterial effect [60]. Additionally, its gas porosity has been used as a gas blocker. [25] It has a low shrinkage stress this means that sensible patterned structures are safe and no sensitivity to oxygen. [8]

Polymers using thiol-ene reactions have the disadvantage in cell culture that if not reacted the thiol groups will bond together to form a disulphide, these bonds are no longer a viable focal point for the hydrogel to attach [32]

Through this chemistry bigger objects (hydrogels) can be bonded with the smaller units (OSTE-PDMS thiol groups) through click-chemistry giving the capability of modifying the surface in one step. [8, 25, 52]

2.7. Surface Characteristics

Cell adhesion in microfluidic devices is achieved by surface treatments with different materials such as natural proteins such as collagen [61] laminin and fibronectin [29, 55]. ECM proteins and molecules can be used to functionalize the substrate material to be more biocompatible [29] The ECM is the key in the cell-ECM interface, it is in there where mechano-sensing of the environment is received by the cells. [13]

For the PDMS to be practical it needs to be biocompatible and support cell adhesion, but cells will only adhere to PDMS when the surfaces are treated with a chemical, ozone, or oxygen Plasma. [53] Other surface modification techniques can be seen in **Figure 2.12**. In the continuous search, different technologies can be used for achieving functionalization of the surface bringing unprecedented level of control in the surface, that will give a robust bioanalytical device for recognition of specific behaviours of cells [26]

There are several techniques to modify the surface of PDMS, but the surface becomes hydrophobic again. Grafting and embedding techniques include the use of a coating on the surface [26]

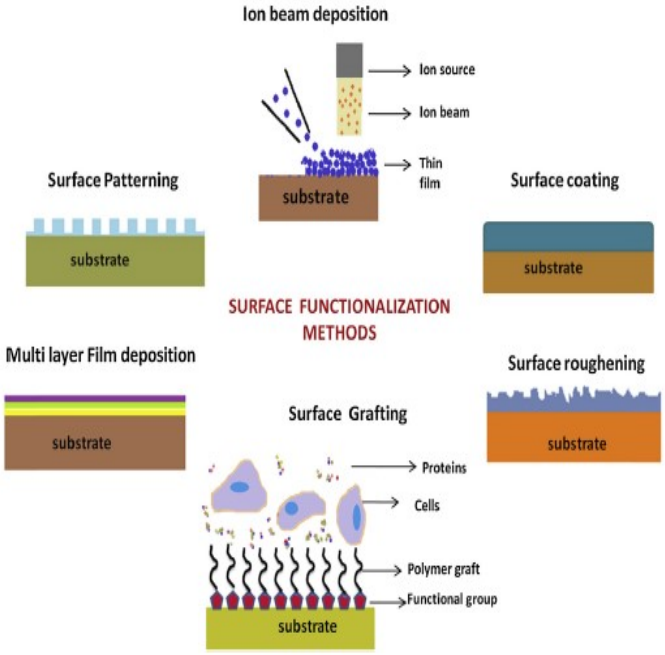

Figure 2.12: A diagram representing different techniques to functionalize the surface of the substrate. [49]

For PDMS in biomedical microfluidic devices, it needs to have its properties modified. Surface modifications will give the material mechanical properties such as hydrophilic surface, conductivity, coatings on its surface for inhibiting ECM binding. Bulk modification to the substrate will alter properties such as elasticity, thermic and electric conductivity. [59]

Elastic Modulus and stiffness of tissues vary in the body; **Figure 2.13** shows the different goals the materials should have to be able to be used and taken under consideration when designing a microdevice. Stiffness is a characteristic of the material that helps us understand the ability of the substrate to manage mechanical forces applied to it. [29]

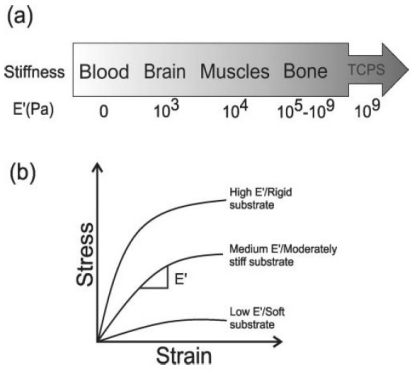

Figure 2.13: a) Stiffness representative chart of different cells and b) Stress vs. Strain curve to obtain the slope called Young's Modulus of the material. [29]

Young's Modulus and the mechanical properties of PDMS demonstrated a transition from showing properties as a bulk substrate to showing properties of thickness-dependent behaviour in the range of 200 µm [59]

Stiffness is a very important characteristic to look for in a substrate material. Cells sense this microenvironment and influence their behaviour; different responses can be expected such as migration, proliferation, focal adhesion. [29] To understand this, in **Figure 2.14** the stiffness affects the Fibroblasts over time. We can see that stiff substrate was preferred by the cells as they spread the most distance in this substrate and showed better adhesion too. Additionally, we can see that the soft to stiff material is showing similar characteristics as the stiff materials; this evidences that the cells can spread better in this surface. In comparison, the soft material shows the worst results compared to the other two. [61]

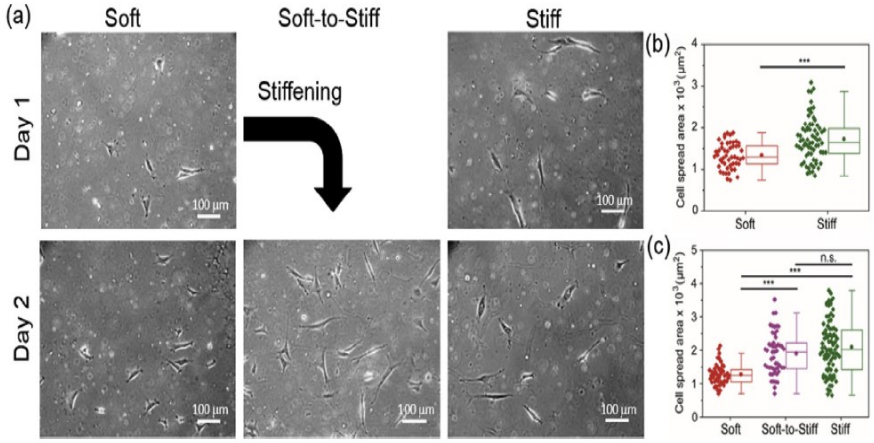

Figure 2.14: Fibroblasts cell proliferation on a soft, stiff and soft to stiff surface, [61] Myofibroblasts differentiation [11, 39, 44]

The stiffness can be tuned by altering the curing time, changing relations of base agents, using different PDMS, embedding biomolecules [19, 44, 59] obtaining substrates liquid or solid-like as required. [61]. Additional studies have shown the Young Modulus is also influenced by curing temperature and reaction time [61]

In a study by [62] it was shown that the morphology and structure of the cytoskeleton of fibroblasts, endothelial cells and neutrophils also show similar results when working with cell cultures on soft or stiff materials but the study also notes that these differences shown by the cells will disappear when allowing cell to cell contact. As the density of cells is major, this kind of contact is a factor for determining morphology and its stress fibers. This supports the idea that mechanical cues can trigger different behaviour in different cells.

A study by [13] has shown that adhesion of the ECM to the substrate is dependent on the stiffness too, showing that stiffer materials enhanced adhesions while softer materials the focal points slipped.

Different factors will determine cell fate, in the beginning, it was believed that the growth factors were the only factor that controlled the cell functionalization, but cell to cell interaction, interaction with the substrate and mechanical forces are also key factors to give a cell the best-simulated microenvironment. [1]

The mechanical environment is a very potent factor and crucial for the cell to grow and differentiate. The key to this mechanical environment to be transferred to the cell is by using flexible and elastic gels to allow cells to form their shape and establish connections with their neighbours.[27] In contrast, growth factors and molecules are used to promote cellular responses and gene expressions [13] Dynamic surfaces are key to provide the space where the biomaterial can interact with living cells and mimic its environment *in vivo*. [49]

2.8. Cell Adhesion

Coatings on the surface of the substrates enhance the cell adhesion and the ECM remodelling. [39] This remodelling is building a network bridge between the membrane and giving focal adhesion points to the cytoskeleton, improving the attachment[13, 57]. This matrix is one of the most common sites for cell adhesion. [62]The ECM matrix is a key protagonist in providing the point for adhesion to the cell and the space for interaction between cells. [1] Additionally, it has been shown by [37, 53] that it also gives temporary hydrophilic characteristics, desired in the device to avoid unwanted absorption of molecules in the system.

Coatings can provide characteristics to enhance cell adhesion by providing a surface which will covalently bind with cell-seeded hydrogels. [38] They have also can be used to improve wettability and non-specific protein binding characteristics of PDMS. [26] The coating needs to be compatible with the reaction of the polymer, to provide a surface modification to enhance cell adhesion. [26] A significant change in surface contact angle of water.

OSTE PDMS is a type of off-stoichiometry thiol-based PDMS that is often used to enhance cell adhesion by providing a thiol-ene reaction in the surface in which will covalently bind with cell-seeded hydrogels. [38] This bond is done by the free groups created by the treatment and allow to covalently bond with the surface.

Synthetic hydrogels are often made of polyethylene glycol (PEG), this material has good properties for its use in OoC technologies as it has characteristics such as customized viscosity, and it can have porosity.[36] Different functional cues can be attached to the polymer via photo cross-linking with UV light. Polyethylene glycol norborene PEG-NB is another type of hydrogel that can be seeded with cell culture. [38] This hydrogel is crosslinked to bond resulting in a thiol-ene reaction. The hydrogel is biodegradable and biocompatible with the body. This material is mostly used because it is one of the most hydrophilic materials and shows a low level of adsorption of proteins. These hydrogels are commonly coated with adhesion peptides to obtain the desired thiol-containing groups to provide the anchoring points to the material. [58]

Figure 2.15 is a device proposed by [38] where the Thiol based PDMS is used in combination with a Thiol containing group hydrogel to be able to covalently bond after the crosslinking is done by UV light. The resulting device is a membrane-based PDMS capable of providing good cell adhesion and a mechanic platform to be able to translate the stimulation directly to the cell culture.

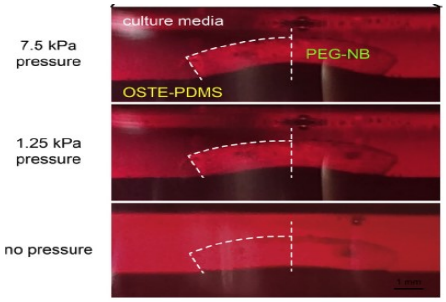

Figure 2.15: Example of an OSTE PDMS bonded with PEG-NB Hydrogel providing a fixed substrate to cell culture. [38]

Cell cultures can be made with embryonic stem cells, primary cells, cell lines, induced pluripotent stem cells (iPSC). [2, 51] Primary cells are functional and mature but are not easy to find and cannot be expanded in culture. Cell lines are easier to expand and simpler to culture, but they will not display functionalities of the organ they intend to mimic. iPSC is an ideal source as they provide an exact footprint of the cells to culture, but not all cell lineages can be obtained from the same IPSC and protocols to differentiate this kind of cells are still under a lot of investigation. [51]

To influence the functionalization of the cell cultures microfluidic devices, use fluid flow and shear stress for differentiation of cells and play an important role in giving the cell its shape [2]. Shear stress will contribute to the development of cells. [19] Mechano-sensitivity enhances cell growth and maturation. [38] Medium perfusion increase functionality. [51]

Cells respond to stimuli in different ways, and environmental cues of many natures can trigger those. [39] **Figure 2.16**. a) Incorporation of biomolecular cues to dictate cell fate [55], b) cellular cultures displayed in 2D rigid substrate vs. a multicellular three-dimensional structure that provides an environment suitable for the cells to migrate and survive. [3], c) mechanical properties will translate directly into the cell affecting its development and adherence, different cues can be perceived by the cell such as stiffness, shear force, and

mechanical load, aligned or randomized networks to proliferate and organized pillar structures with gratings [29]

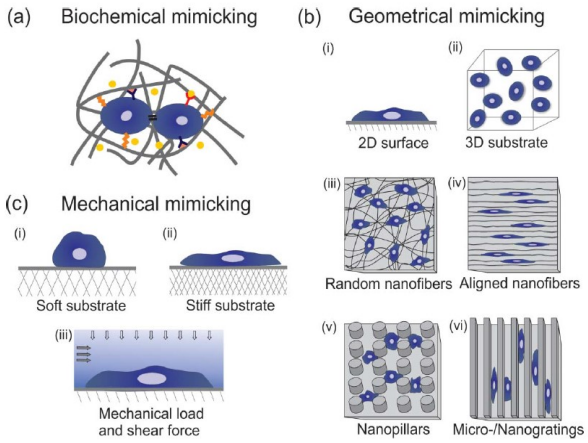

Figure 2.16: A culture of cells with different environmental cues to determine cell fate. [29]

3

Design and Fabrication

This stage of the project is going to be based on the article by Liu [38] where a similar process is followed. This will then be followed by the fabrication process and finally a discussion. To understand the new formulation of OSTE-PDMS we need to understand how an eternally applied force will affect it. These conditions will dictate the material will behave differently. It can behave elastically or plastically. Elastic deformation means the material will be able to recover to its original state and will behave mostly in a linear way. To mechanically analyse it, we need to obtain its properties to be able to characterize it. The most common way to evaluate a material is to perform a tensile test. The test is made to obtain a stress strain curve. In this curve, we focus on the elastic region. [50, 56]

To achieve this curve, the material is cut into specific dimensions and loaded into two clamps. One end is static and the other one will pull with a constant force and simultaneously measure the sample response until a specific strain or until rupture. A material exhibits an elastic behaviour when the deformation produced under an applied force is completely recovered after its removal. This means there's a linear behaviour between its stress and strain, the slope of this curve is called the Young's modulus and is usually measured in GPa. This elastic modulus is a value which speaks about the ability of the material to be deformed by the presence of stress. This value is used to understand how stiff or rubbery a material structure is. [50]

3.1. OSTE-PDMS Formulation

The composition ratio the OSTE-PDMS used during this project is of thiol functionalized PDMS: vinyl functionalized PDMS: vinyl terminated PDMS of 1000, 5000 and 1000. The project uses a formulation of OSTE-PDMS with a ratio of 2:0.3:1.5:1.5:1.5 this produces OSTE-PDMS with a low young's modulus which means it has elastic properties as well as being compatible with fabrication. [14] The ratios have an excess thiol groups making the formulation an off-stoichiometry thiol ene polymer [38]

The OSTE PDMS formulation is mixed with the following components:

- Dimethylsiloxane copolymer with 4–6% (mercaptopropyl) methylsiloxane or thiol-functionalized PDMS (SMS-042),
- 4–5% Vinylmethylsiloxane or vinyl-functionalized PDMS (VDT-431),
- vinyl-terminated PDMS (DMS-V31, DMS-V35 and DMS-V41 with viscosities of 1000, 5000 and 10,000 cSt, respectively)
- photoitiator TPO-L

Once the crosslinking of the polymers is done and the curing is complete the resulting polymer has a elasticity lower but with similar elastic behaviour as PDMS. OSTE-PDMS can also customize its mechanical properties by altering the ratios of vinyl-functionalized PDMS to vinyl-terminated PDMS, the current ratio use is 5:1 which means there are five times more vinyl terminated than vinyl functionalized PDMS. This OSTE-PDMS composition ratio a of 2:0.3:1.5:1.5:1.5 is able to produce a polymer compatible with deformation, but

also compatible with the device fabrication. All the polymers share one characteristic, and that is that all of them are PDMS, the difference between them is that each PDMS has a different functional group attached to it. This formulation produces a passive permanent surface for thiol reactive molecules to be able to bond physically and stay in place ensuring a good adhesion between the interfaces of the device.

3.2. Tensile Testing

The tensile moduli of OSTE-PDMS samples were characterized using a commercial mechanical test machine Dynamic mechanical analysis (DMA). For tensile testing, samples were cut into rectangles of 2cm x 5cm x 1mm of OSTE-PDMS slabs with two samples per composition ratio. Each sample was stretched up to 40% of strain to be in the elastic area. The Young's modulus was obtained by calculating the slope in the linear portion of the stress–strain test. This linear relation is given by Hooke's Law

$$\sigma = E\varepsilon$$

Where σ is the applied stress, E is the constant of proportionality and ε is the strain. The stress-strain curves are shown in **appendix-b**. Compared to a 5:1 standard PDMS the elastic modulus of OSTE-PDMS reaches 140 KPa with a strain of 40%, which is much lower than the 558kPa of the PDMS. In comparison to the OSTE-PDMS control group and OSTE-PDMS with the two hydrogels: values of 100, 120 and 140 KPa respectively, there's evidence that the excess of thiol groups in the formulation can contribute to increase the modulus, this means the tensile strength and elongation can be higher. The value obtained for the OSTE-PDMS in the laboratory is not similar to the one obtained by [38] in the literature 284kPa. One of the reasons this is believed to have happened is that there's a possibility that the polymer was slipping from the DMA holder due to its very flexible, soft and elastic nature.

In **Figure 3.1** and **Figure 3.2** are examples of the tensile test performed in TU Delft with a DMA. These figures show both the testing of the hydrogel control OSTE-PDMS and the bonded hydrogel OSTE-PDMS

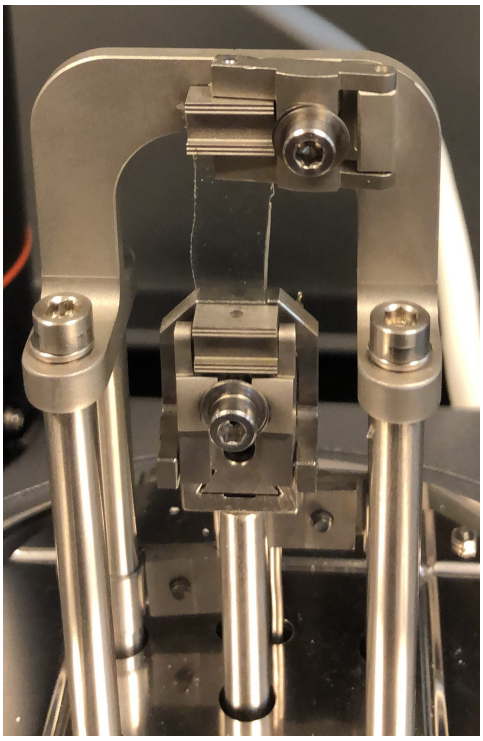

Figure 3.1: Example of the OSTE-PDMS control tensile test. Taken from Dynamic Mechanical Analysis tool.

Figure 3.2: Example of the OSTE-PDMS + hydrogel tensile test. Taken from Dynamic Mechanical Analysis tool

3.2.1. Results PDMS

In **Table 3.1** and **Table 3.2** the Young's modulus was obtained on PDMS 10:1 ratio of curing to base agent and second sample with a ratio 5:1 Using Dynamic Mechanical Analysis tool the samples were tested and analysed to compare them to OSTE-PDMS formulation. The 10:1 ratio was characterized due to the fact that most of the literature uses this ratio. The 5:1 ratio was analysed to directly compare it to the OSTE-PDMS formulation which follows a 5:1 ratio in its Vinyl terminated to Vinyl functionalized which were reported by Liu [38] to be the ratios to use to increase the stiffness of the sample. Finally, a 20:1 PDMS which has a similar Young's Modulus as the OSTE-PDMS

Standard PDMS measured curves have lower but similar values than literature.[45] This is due to the fact that these materials are very flexible and the clamping tool may have slipped. There needs to be a balance with the amount of force used to maintain the sample in place but at the same time not applying a lot of force because it can deform the sample.

Table 3.1: Elastic results PDMS 5:1

Properties	Value
Young's modulus (KPa)	825.27

Table 3.2: Elastic results PDMS 10:1

Properties	Value
Young's modulus (KPa)	540.68

Table 3.3: Elastic results PDMS 20:1

Properties	Value
Young's modulus (KPa)	97.62

3.2.2. Results control OSTE-PDMS

In **Table 3.4** the Young's modulus of a single OSTE-PDMS sample was measured. This test was meant to be used as a control to analyse if the hydrogel bonding contributes to its stiffness. The test performed in TU Delft gave a result of 107KPa which is different than the one reported by Liu [38] this may have been to the fact that the six component formulation is very hard to get exact and small variations may change the outcome of the polymer. Regardless, in both of the literature and in practice a very soft solid polymer was obtained that was compatible with the fabrication process

Table 3.4: Elastic results control OSTE-PDMS

Properties	Value
Young's modulus (KPa)	107.5

3.2.3. Results OSTE-PDMS with Hydrogel DT

In **Table 3.5** we measured a sample of OSTE-PDMS with UV cured hydrogel bonded to the surface through a process that will be explained in the next chapter. In these results we can directly compare it to the control. The sample containing a fresh hydrogel is able to make the material stiffer by increasing its Young's modulus from 107kPa to 120kPa. The measured value differs from Liu [38] but in this case both of the articles show that with a hydrogel bonded to the surface the Young's Modulus is higher.

Table 3.5: Elastic results OSTE-PDMS bonded with hydrogel DT

Properties	Value
Young's modulus (KPa)	120

3.2.4. Results OSTE-PDMS with hydrogel ODT

In **Table 3.6** we measured a chemically induced hydrogel bonded to the surface of the OSTE-PDMS. The Young's modulus of 140kPa obtained for this sample is the highest obtained comparing it to the control OSTE-PDMS and the UV cured hydrogel. This shows that a milder reaction such as the one carried by this chemistry will have a hydrogel that contributes a 40% stiffer sample.

Table 3.6: Elastic results OSTE-PDMS bonded with Hydrogel ODT

Properties	Value
Young's modulus (KPa)	139

3.2.5. Viscoelasticity

Polymers are elastomers which means they have a certain viscoelasticity. These materials can show a viscous and elastic behaviour. For this reason this material can be seen as combination of both behaviours. [17][50] Using the dynamic mechanical analysis (DMA) with a sinusoidal rate instead of a constant force in one direction can measure both elastic and viscous properties of the material; at the same time. [17] The storage modulus represents the real part energy stored in the region where the material behaves elastic its often compared to the Young's Modulus because they can be seen as the stiffness but they are not comparable in value . The loss modulus represents the viscous or imaginary part this part will tell us how much of this energy is being lost to the viscous part. The sum of loss and storage modulus is called complex modulus G*. The complex modulus is conformed by:

$$E(\omega)^2 = E(\omega)'^2 + E(\omega)''^2$$

[17] If the storage modulus value is higher than the loss modulus value the material will behave mainly elastically.

In **Table 3.7**, **Table 3.8**, **Table 3.9** and **Table 3.10** we have the information gathered by an oscillatory test on the different samples. Using this tool, we have confirmed that in a OSTE-PDMS and a standard PDMS sample the storage modulus is higher than the loss modulus which means that both of the materials can be regarded as elastic materials. The Storage modulus is associated with the elastic part of the curve and is considered the real part. It's often related to creep and stress relaxation. The Loss modulus is associated with the viscous behaviour and is considered to be the imaginary part.[42]. It's often related to hysteresis and internal friction of the material. The Loss modulus usually manifest itself as dissipated energy heat and it can be seen as damping. In this measurement the storage modulus is not exactly the same as the Young's modulus reported in the previous test. This is believed to be because of a bad curing of the sample. The loss modulus in the OSTE-PDMS means that this material will be damaged through time because of its viscous behaviour.

Table 3.7: Viscoelastic results PDMS 10:1

Properties	Value
Storage Modulus(KPa)	1100
Loss Modulus (KPa)	199
Complex Modulus (KPa)	1117.86

Table 3.8: Viscoelastic results PDMS 5:1

Properties	Value
Storage Modulus(KPa)	1500
Loss Modulus (KPa)	400
Complex Modulus (KPa)	1552.42

Table 3.9: Viscoelastic results PDMS 20:1

Properties	Value
Storage Modulus(KPa)	155
Loss Modulus (KPa)	68
Complex Modulus (KPa)	169.26

Table 3.10: Viscoelastic results OSTE-PDMS 2:0.3:1.5:1.5:1.5

Properties	Value
Storage Modulus(KPa)	51
Loss Modulus (KPa)	56
Complex Modulus (KPa)	75.74

3.3. Fabrication

During this stage of the project the micropatterned thin membrane made of OSTE-PDMS will be created in order to be able to provide a passive surface activation where a thiol reactive hydrogel can be bonded to. The process will be exposed as follows:

1. Back side patterning
2. OSTE-PDMS coating
3. Metal Deposition
4. Patterning of the OSTE-PDMS
5. Etching metal
6. Etching polymer
7. Release of the membrane
8. Final device

A cross section of the creation of a porous membrane fabrication process is exposed in **Figure 3.3**

3.3.1. Back-side Patterning

The first steps in the process include the use of a 4" double side polished (DSP) silicon wafer and begin the creation of the zero layer which contains the alignment marks etched inside the surface of the silicon wafer itself, and deposit SiO_2 with plasma enhanced vapor deposition (PECVD) a 2000nm layer of in the front and a 5000nm layer in the back. After those initial steps are done the first stage of the process is to use lithography to introduce the first pattern to be transferred to the back of the wafer. Positive resist is coated in the surface of Si wafer and a mask with 52 circles a dimension of 5mm of diameter and a distance of 1cm between each circle is exposed with UV light and developed. This pattern is where the trenches will be created to suspend the OSTE-PDMS membrane. Next step is to remove the SiO_2 and expose the SI inside the area of the circle. **Figure 3.4** is a picture taken inside the clean room with a pattern of 52 individual circles of 0,5mm of diameter with 1 cm of separation between each circle. The pattern in the picture is placed on top of PDMS to calculate the area of membrane required.

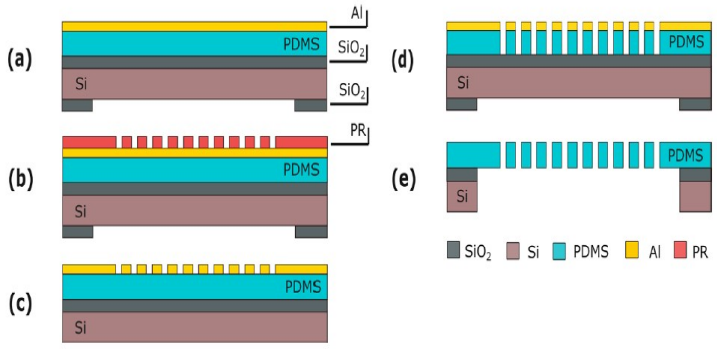

Figure 3.3: Micro-pore array for the membrane [19]

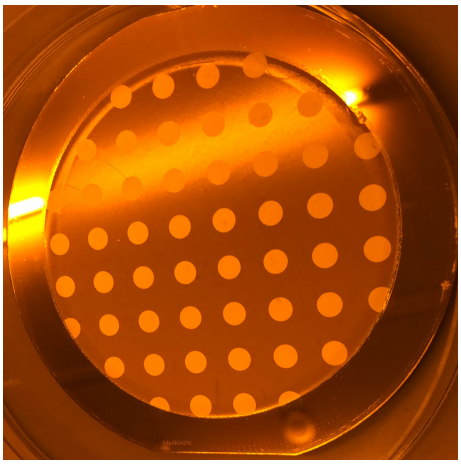

Figure 3.4: Circular windows patterned on the back of a 4" Si wafer on top of PDMS for dimensions of the thin membrane

3.3.2. OSTE-PDMS Coating

After the patterning of the windows is completed, next stage is to spin coat a thin membrane of OSTE-PDMS on top of 2000nm of SiO_2. The coating pm top of the Si wafer is done with a recipe consisting of 10 seconds spinning with a speed of 500rpm followed by a second step of 2500rpm for 42 seconds producing a membrane of 8 μm. Special attention is required due to degassing in the material.**Figure 3.5** is an example of a OSTE-PDMS cured membrane. Material is removed from the edge of the silicon until the exposure of the alignment marks. This step is required due to impossibility of using the alignment marks if they are under the OSTE-PDMS membrane. The deposition of SiO_2 serves the purpose of providing a landing point to the OSTE-PDMS, and will be the only material separating the OSTE-PDMS porous membrane once the silicon windows on the back of the wafer are removed. Once the OSTE-PDMS is on top of the Si wafer the OSTE-PDMS needs to be exposed to a UV light for it to change from a liquid stage to a solid flexible thin membrane. The OSTE-PDMS is exposed for 6 minutes under a 5,5mj/cm^2 intensity UV light to obtain a cured OSTE-PDMS membrane. Special steps such as using a desiccator and baking the membrane in the convection oven are used techniques during the process for the degassing of the polymer. These are further discussed at the end of the chapter in the discussion section.

3.3.3. Metal deposition

This step requires to have a leak up test rate made before each deposition, if the rate test is failed, placing the membranes in the oven has shown to eliminate all left-over solvents present in the membrane. Once the leak up test is passed the process can continue to deposit a 200nm Aluminium layer on top of the OSTE-PDMS as the **Figure 3.6** shows us. Sputtering is a Physical vapor deposition (PVD) procedure where the OSTE-PDMS is placed under vacuum and the sputtering target (metal) is placed on top of the Si wafer. Using an inert gas and

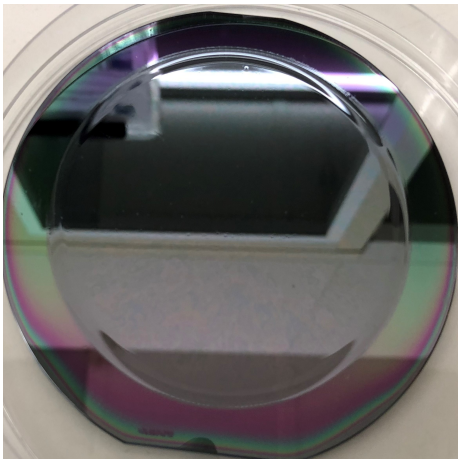

Figure 3.5: A thin membrane of OSTE-PDMS on top a 4" silicon wafer

a Radio Frequency generator the gas is ionized. These ions will accelerate with direction to the OSTE-PDMS with such intensity that the ions from the target are released as vapor. The vapor will condensate in the surface of the wafer. This layer serves as a hard mask for protection of the OSTE-PDMS as well as providing a flat surface for the patterning to be transferred.

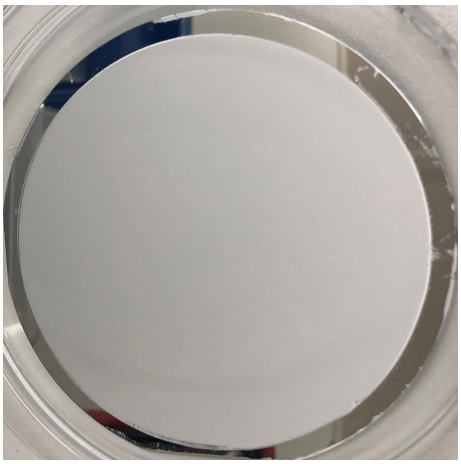

Figure 3.6: Aluminium deposition on top of OSTE-PDMS membrane

In **Figure 3.7** taken with Keyence VK-X200 Series Laser Microscope with a lens magnification of 150X, stress marks are present in the surface of the metal layer even before the patterning stage was performed (which use high temperature baking of the wafer). This can be a consequence of the baking of the cured OSTE-PDMS used to evaporate its remaining solvents. Degassing will be discussed later in this section.

3.3.4. Patterning of the OSTE-PDMS

From this step forward the process needs to avoid quick temperature swings due to the different thermal expansion coefficients between the OSTE-PDMS and the Aluminium layer. Custom coating recipes that omit baking steps are used during this phase. The photoresist used in this step is a negative photoresist, this is due to the fact that this is the best choice for lift-off processes. The negative resist will help to prevent the coating of the side walls and will be easier to develop. After exposing, the photoresist requires baking at a temperature of 115°C. for crosslinking, after this, during the developing process the photoresist surrounding the circle pattern will stay, leaving the area inside the circle soluble to the developer. **Figure 3.8** is a picture of the mask used for this project. This mask is designed to create micropores in the surface of the OSTE-PDMS.

Figure 3.7: Image of stress marks after Aluminium deposition. Picture taken with Keyence VK-X200 Series Laser Microscope

This design is made of small circles of 10 μm in diameter separated by 20 μm from each other. By using a negative resist, the inside of the circle will be washed out, making it reactive for the metal etching

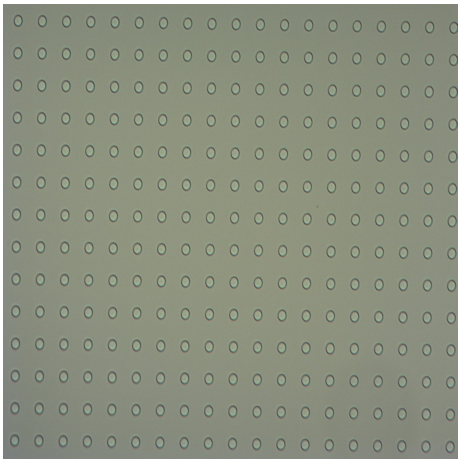

Figure 3.8: Mask used to pattern the pores. Circles of 10 μm in diameter separated by 20 μm. Picture taken with optical microscope with 20X magnification

In **Figure 3.9** we can see how the design in the mask is transferred to the metal layer. In the image there are clear signs of stress marks in the layer. These are suspected to have appeared during the crosslinking bake of the negative photoresist, there's a difference in the coefficients of thermal expansion between the metal layer and the polymer membrane. The stress is transferred to the metal because the OSTE-PDMS is expanding much faster than the metal layer.

3.3.5. Etching metal

The next phase in the fabrication process is to etch the top of the pore. The distribution of the developed photoresist is key in this step as it protects the surrounding of the pore while letting the metal inside the area of the circle be removed from the layer, exposing the polymer underneath the metal to be etched. In**Figure 3.10** and **Figure 3.11** the OSTE-PDMS membrane is exposed by removing the metal layer. The space looks black because of the impossibility of the light to reflect any image. It's suspected that the surface has become very rough making the scattering of the light very big and difficult to travel.

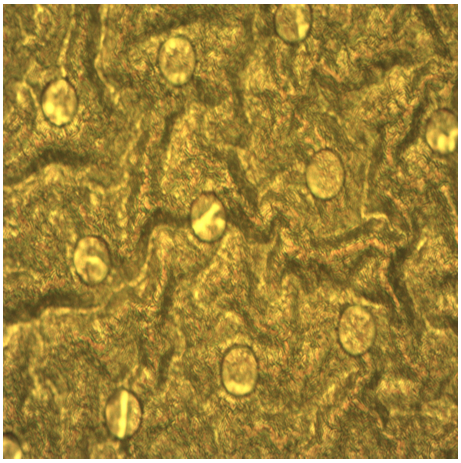

Figure 3.9: Pattern of the pores. circles of 10 μm in diameter separated by 20 μm on top of Aluminium. Picture taken with optical microscope with 50X magnification

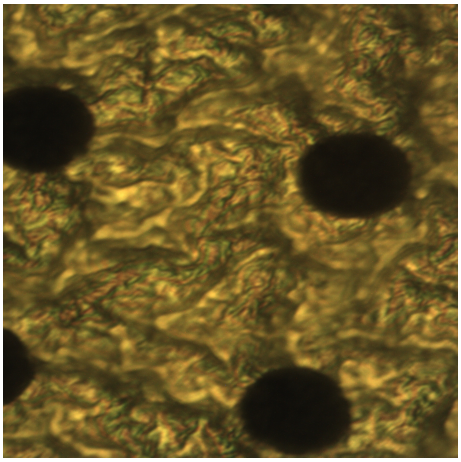

Figure 3.10: Etching the metal on top of the pore. circles of 10 μm in diameter separated by 20 μm on top of Aluminium Picture taken with optical microscope 50X magnification

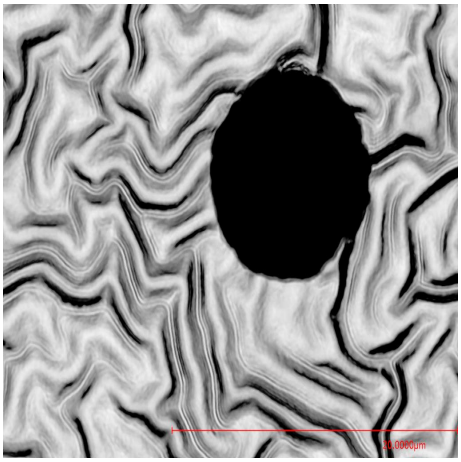

Figure 3.11: Etching the metal on top of the pore with a Laser 150X 3X Zoom circles of 10 μm in diameter separated by 20 μm on top of Aluminium. Picture taken with Keyence VK-X200 Series Laser Microscope

3.3.6. Etching polymer

After removing the top of the pore, the next step is to remove the OSTE-PDMS inside the pore. This step is the longest and most difficult step during the process. To perform the etching we use Inductively Coupled Plasma Etching (ICP) mainly with CF_4 and SF_6 gases to build a fluorine based plasma. Fluorine is a radical which will chemically react with the Si inside of the OSTE-PDMS. One problem with the reaction is that residues such as fluorocarbon can deposit in the chamber and the surface of the wafer. This causes surface roughness. The inability to look inside the pores with any optical tool makes it difficult to have a good idea of the etching rate of the OSTE-PDMS. For this step it took 44 minutes to etch 8 μm of polymer, inside a 10 μm circle. In **Figure 3.12** and **Figure 3.13** there's an image of the pore after removing all the material from inside landing on the 2000nm SiO_2 layer.

This step performed in standard PDMS during Quiros Solano [48] articles showed that for a 10μm of standard PDMS 33 minutes are required to etch away the material vs 44 minutes of the OSTE-PDMS. The use of 6 components to perform the OSTE-PDMS formulation makes the chemistry inside the polymer different than standard PDMS which may be the reason for the increase in etch time. The etch rate is area dependant, which means that the removal of the material and the reactive ions and radicals are creating a small "traffic jam" due to the size of the pores. Another reason that may be causing this increased etching rate is that the formulation has a low Young's Modulus comparable to a 20:1 standard PDMS. 20:1 PDMS compared to 10:1 takes a longer time to etch in practice.

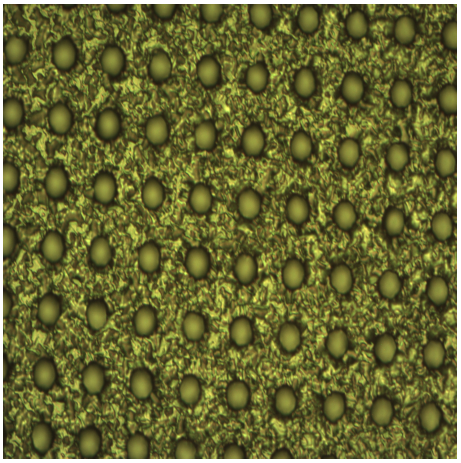

Figure 3.12: Complete etching of OSTE PDMS. circles of 10 μm in diameter separated by 20 μm on top of Aluminium. Picture taken with optical microscope with 20X magnification

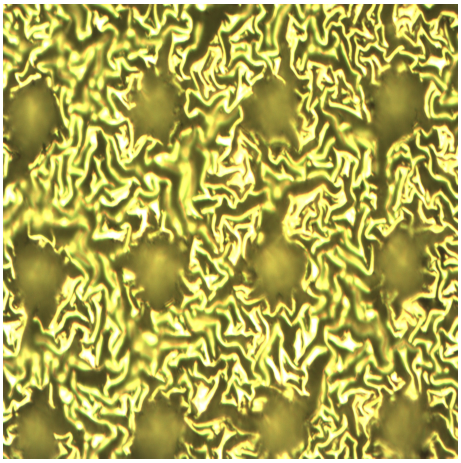

Figure 3.13: Complete etching of OSTE PDMS. circles of 10 μm in diameter separated by 20 μm on top of Aluminium. Picture taken with optical microscope with 50X magnification

Using a laser microscope such as the Keyence VK-X200 has big advantages, the definition of the details with a laser microscope is higher than optical microscopes. This tool has the capacity of building a 3D model of the sample, this can be seen in **Figure 3.14** and **Figure 3.15**, These images help us to have an idea of the height profile of the pore in the membrane. Although it does give a detailed image, the size of the pore in this particular project and the roughness in the surface inside of the pore makes it hard for the tools to have a good and accurate image of the inside of the pore. But still it does give enough information to see metal layer on top of the membrane with some damage. To see a better image the use of a powerful tool such as a SEM.

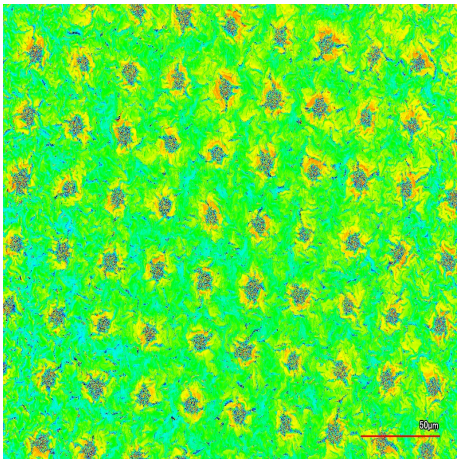

Figure 3.14: 3D Height profile of OSTE-PDMS pores. circles of 10 μm in diameter separated by 20 μm on top of Aluminium. Picture taken with Keyence VK-X200 Series Laser Microscope

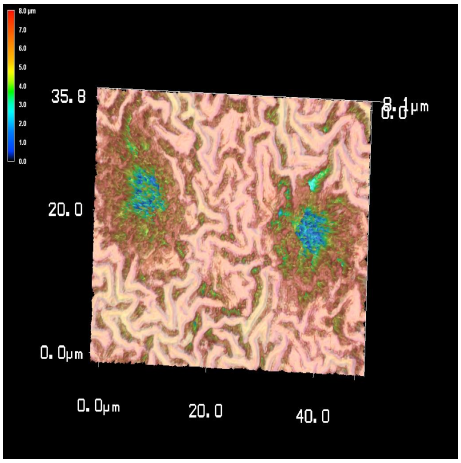

Figure 3.15: Height profile of OSTE-PDMS pores Using magnification 150X 3X Zoom. Pores of 10 μm in diameter separated by 20 μm on top of Aluminium. Picture taken with Keyence VK-X200 Series Laser Microscope

SEM microscopes help us to have a high resolution and highly confident image by scanning the surface of the sample with a focused beam of electrons. These particles interact with the atoms inside of the sample and produce a signal which is decoded and used to form the image. In **Figure 3.16**, we can appreciate how the pores and the metal layer look like, from there we can zoom in to see the details of one pore. **Figure 3.17** and **Figure 3.18** are examples of images of a completed pore.

The images taken in **Figure 3.19** and **Figure 3.20** are examples of a cross section of the membrane. These

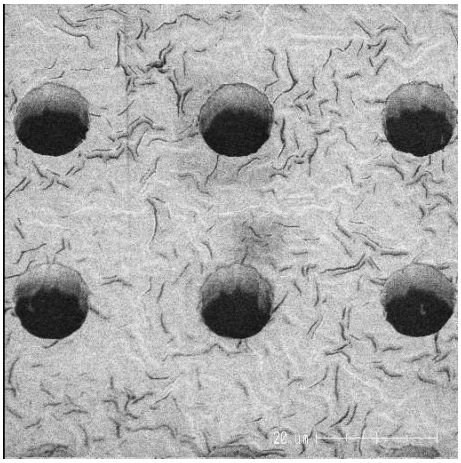

Figure 3.16: Example of a 8 μm in height pore with dimensions 10 μm in diameter separated by 20 μm etched on top of the OSTE-PDMS. Image taken using a SEM

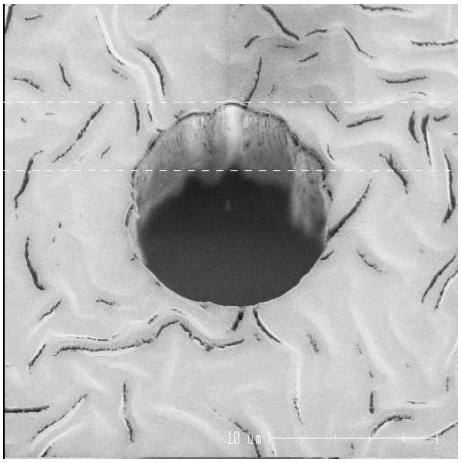

Figure 3.17: Example of a 8 μm in height pore with dimensions 10 μm in diameter etched on top of OSTE-PDMS. Image taken using a SEM

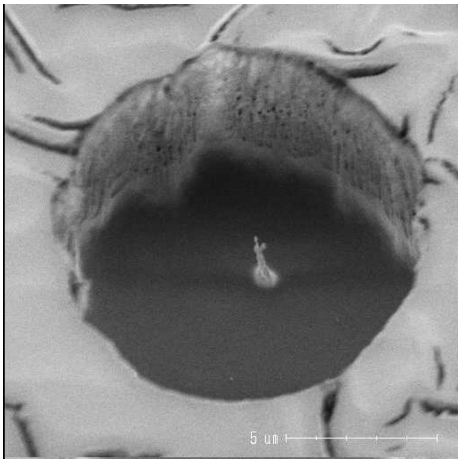

Figure 3.18: Example of a 8 μm in height pore with dimensions 10 μm in diameter etched on top of OSTE-PDMS. Image taken using a SEM

images were obtained by breaking the samples in half to obtain the desired sample. Using this tool, the rate of etching the OSTE-PDMS was calculated to be 180 nm per minute. During the process images such as **Figure 3.19** are examples of a under etched pore, compared to **Figure 3.20** which is a over etched pore, the oxide layer and the silicon under the membrane were etched, and the metal layer on top has evidence of some damage due to ion bombardment. Using these images a time of 44 minutes was chosen to fully etch the polymer, landing in the SiO_2 **Figure 3.12**

In **Figure 3.21** there's a cross section of the device before it continue the process of suspending the membrane.

3.3.7. Release of the membrane

To etch the silicon circles on the back, DRIE (Deep-Reactive-Ion-Etching) is used to etch the 500 μm of silicon. This step uses plasma and ion bombardment. The plasma ionizes the gas and the ion is then accelerated to hit the surface of the target. The new bond created by the bombardment is stronger than the bonds in the surface itself which makes it possible to detach the silicon and pump it out. During the process the Silicon wafer has received enough mechanical stress as well as modification to its surface that a carrier wafer is required to use this tool. A polymer-based glue is used to provide adhesion between the two wafers protection to the tool holding chuck, and to improve the metal conduction between the two Si wafers. The glue is spread in the

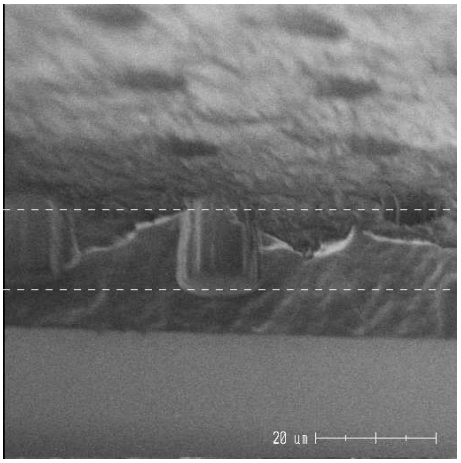

Figure 3.19: Cross section of a porous OSTE-PDMS membrane etched for 27 minutes. Image taken using a SEM

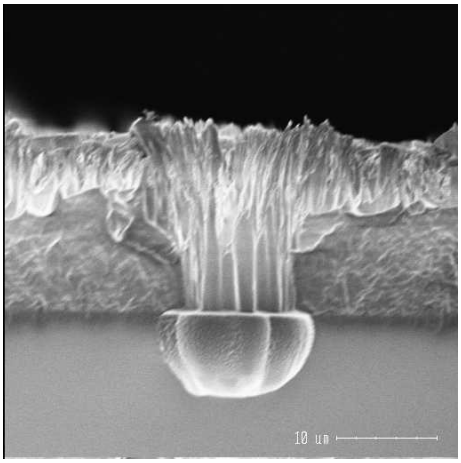

Figure 3.20: Cross section of a porous OSTE-PDMS membrane etched for 52 minutes. Image taken using a SEM

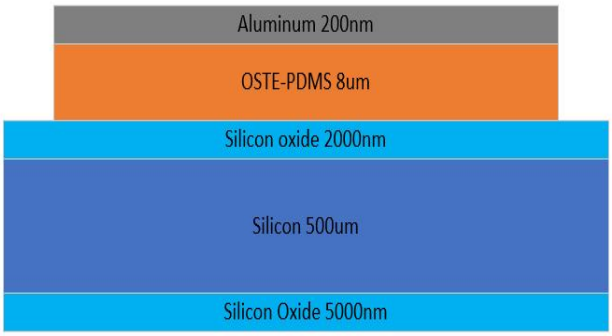

Figure 3.21: Cross section of the layers before the release of the membrane

wafer in a way that ensures many points of contact with the surfaces, the reasoning behind this is to provide a better thermal conduction between layers. A total of 560 cycles were required to completely land in the oxide stopping layer under the OSTE-PDMS membrane. A rate of 892,9 nm per cicle is etched

In **Figure 3.22** we can already see the membranes inside the radial windows, the membranes show a grey colour because of the carrier wafer still being under the device. This picture can be seen with better detail in **Figure 3.23** and **Figure 3.24**. The carrier wafer has been detached using Isopropyl alcohol. The images were purposely placed in the border of the suspended membrane, in the picture the contrast between both layers is clear, the suspended membrane is showing a black colouring on the inside because of the colour of the holding chuck of the microscope. All of the previous three images still contain an aluminium layer on top of the membrane as well as the 2000nm of SiO_2 under the membrane.

3.3.8. Removing the SiO_2 and Aluminium

Once the suspended membrane has been achieved, the final step of the process is to etch the remaining 2000nm of SiO_2 under the membrane and the 200nm of Metal layer on top of the OSTE-PDMS membrane. To do this a procedure known as wet etching is used for 10 minutes, buffered oxide etch (BHF) is used in microfabrication industries to have a very controlled etch of a thin oxide layer. After the etching of the oxide has ended a rinse for 5 minutes in distilled water is required. Finally, the last step would be to remove the remaining metal layer on top of the OSTE-PDMS membrane this is done using a mixture Phosphoric, Acetic and Nitric acid for 2 minutes.

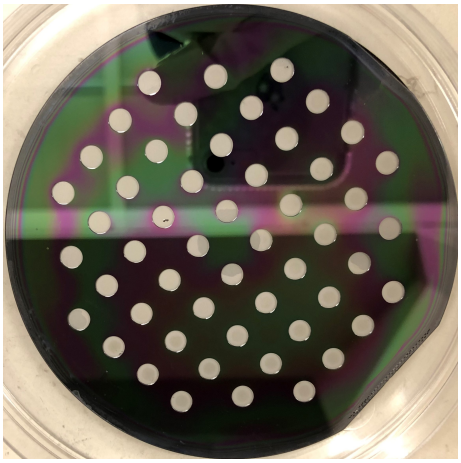

Figure 3.22: Opening of the Si pattern on the back. Circles of 5mm in diameter separated by 1 cm

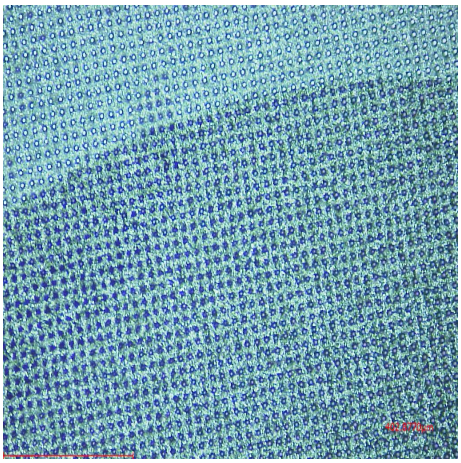

Figure 3.23: Contrast of the edge of the suspended membrane and the silicon. Porous membrane still containing oxide layer and metal on top. circles of 10 μm in diameter separated by 20 μm on top of Aluminium. Picture taken with Keyence VK-X200 Series Laser Microscope 10x magnification

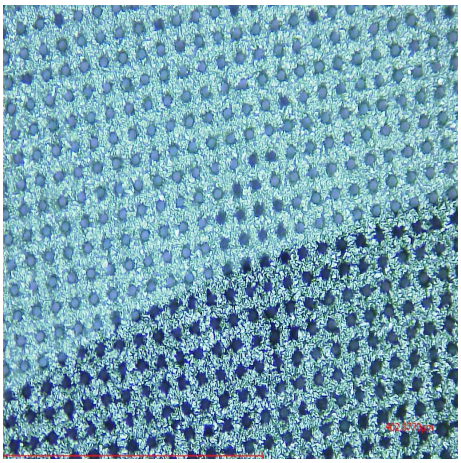

Figure 3.24: Contrast of the edge of the suspended membrane and the silicon. Porous membrane still containing oxide layer and metal on top. circles of 10 μm in diameter separated by 20 μm on top of Aluminium. Picture taken with Keyence VK-X200 Series Laser Microscope with 20X magnification

In **Figure 3.25** and **Figure 3.28** we can see an example of a device with membrane of OSTE-PDMS suspended. This picture contains an un-patterned suspended membrane used as a control device when performing testing on the hydrogel bonding.

A complete finished fabrication can be seen in both **Figure 3.26** and **Figure 3.27** in the latter picture evidence of damage to some membranes can be seen. This damage is suspected to have happened during the carrier wafer removal, although alcohol was used to dissolve the polymer glue, some of the membranes suffer some damage due to the force required to slide the carrier wafer out of place.

In **Figure 3.29**, **Figure 3.30** we see an example of the final device under the microscope without the oxide layer and the metal layer on top. Compared to **Figure 3.23** and **Figure 3.24** where the device still had the layers still on top. We can see how the suspended membrane in **Figure 3.30** show some details of flexibility.

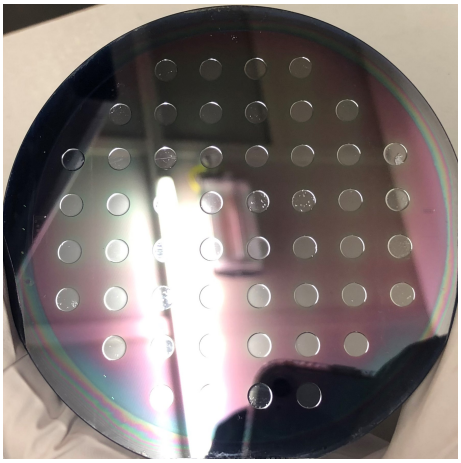

Figure 3.25: Suspended un-patterned OSTE-PDMS membrane. Circles of 5mm in diameter separated by 1 cm

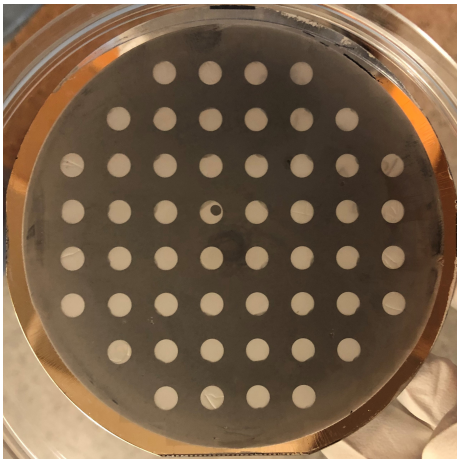

Figure 3.26: Example of a suspended porous OSTE-PDMS membrane. Windows of 5mm in diameter separated by 1 cm with porosity of 5 μm in diameter separated by 20 μm

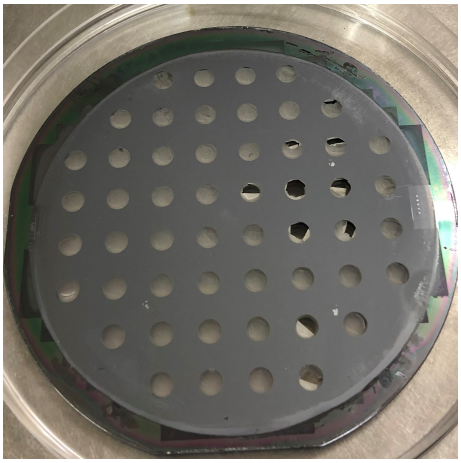

Figure 3.27: Example 2 of a suspended porous OSTE-PDMS membrane. Openings of 5mm in diameter separated by 1 cm with porosity of 5 μm in diameter separated by 20 μm

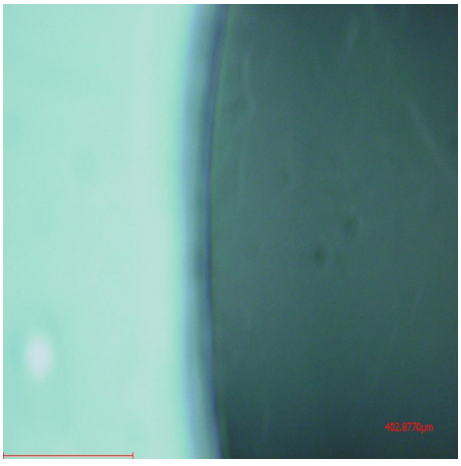

Figure 3.28: Suspended un-patterned OSTE-PDMS membrane. Openings of 5mm in diameter separated by 1 cm. Picture taken with Keyence VK-X200 Series Laser Microscope 10x magnification

3.4. Discussion

3.4.1. Stress marks

The chosen method for degassing the membrane is the reason why the metal layer is showing some stress marks. As showed in **Figure 3.31** the OSTE-PDMS surface has been modified and is showing some wrinkles

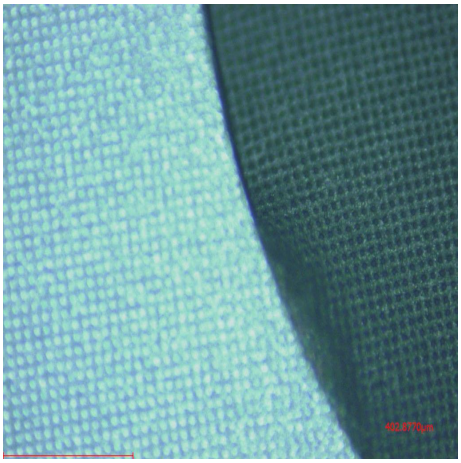

Figure 3.29: Example of a suspended porous OSTE-PDMS membrane. Openings of 5mm in diameter separated by 1 cm with porosity of 5 μm in diameter separated by 20 μm. Picture taken with Keyence VK-X200 Series Laser Microscope 10x magnification

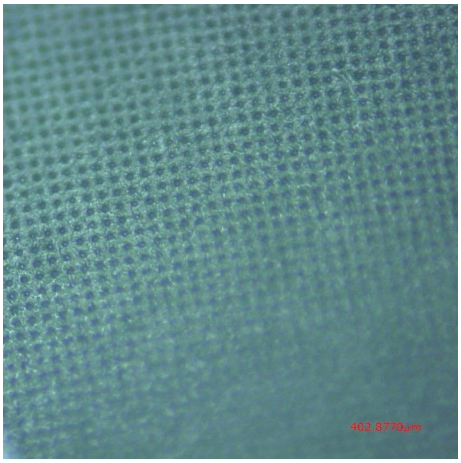

Figure 3.30: Example 2 of a suspended porous OSTE-PDMS membrane. Openings of 5mm in diameter separated by 1 cm with porosity of 5 μm in diameter separated by 20 μm. Picture taken with Keyence VK-X200 Series Laser Microscope 10x magnification

in the surface. These wrinkles are only shown with a high magnification and they don't interfere with the transferring of the pattern. Another possible reason is that during the metal deposition the tool sometimes was heated above 25°C this caused the OSTE-PDMS membrane to expand, the metal layer will be deposited on top of the expanded membrane and when its cooled off the membrane will shrink and will cause wrinkles to show in the metal layer.

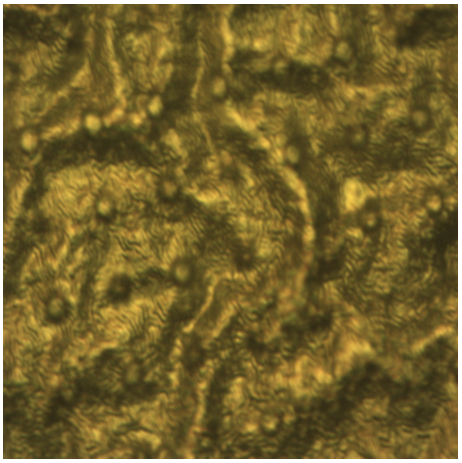

Figure 3.31: Stress marks transferred to the metal layer. Picture taken with optical microscope with 20X magnification

3.4.2. Thermal Shock

In addition to the stress marks in the surface of the metal layer. The process is required to avoid all sudden and abrupt changes in temperature, such as the ones done in the coating and developing process. In **Figure 3.32** and **Figure 3.33** the temperature from the baking of the photoresist has caused some cracks to show on the metal layer. The heat during the whole process is kept under 95°C. The only required step to be in a temperature above that is the crosslinking stage of the negative photoresist which is for 7 minutes at 120 °C., To avoid any abrupt change in temperature a big convection oven is used to transfer the heat slower to the silicon wafer

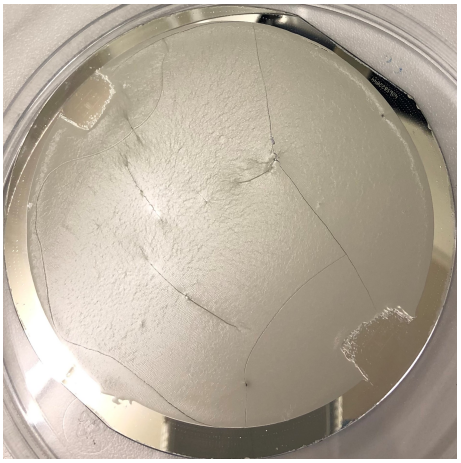

Figure 3.32: Cracks formed in the metal layer.

Figure 3.33: Cracks formed in the metal layer

3.4.3. Degassing

The formulation of a polymer involves the mixing the base and curing agent this causes a chemical reaction releasing bubbles inside the uncured formulation. These bubbles impact negatively the performance of the formulation in its strength and transparency. Therefore, degassing the formulation of polymers becomes an important requirement. This process was done in three different manners, all of them use the Leak Up test rate as the measure to determine which degassing method is better.

The first method is the use of a mixing and degassing tool. The way this tool works is to begin with 500 rpm in its rotor for 30s, during that stage the mixing of the fluids is done. Following the mixing, the rotor speed accelerates until 2500rpm and maintains its speed for 2 mins. This method sometimes proves to be enough, but during the fabrication process degassing issues appeared

During coating the wafers experience baking steps, in **Figure 3.34** and **Figure 3.35** we can see how the metal layer has been damaged by the degassing of the polymer membrane under it. The gasses under the metal layer are trying to escape when the wafer is heated by the recipes.

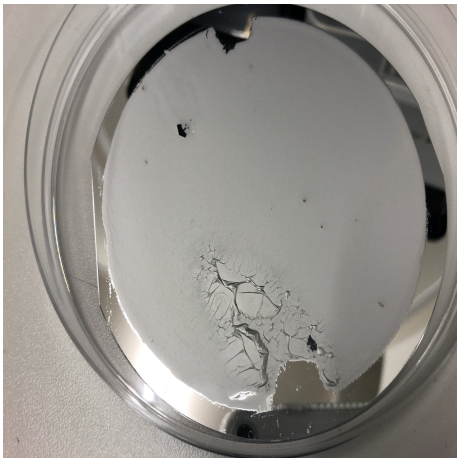

Figure 3.34: Issues caused by degassing o the OSTE-PDMS

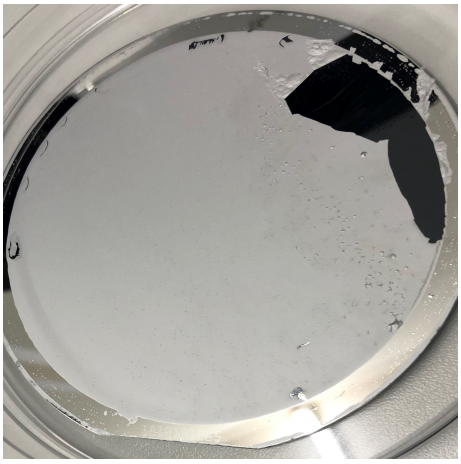

Figure 3.35: Issues caused by degassing o the OSTE-PDMS

To improve the Leak Up Rate of the material two methods were tried. The first method was to use a desiccator with a uncured OSTE-PDMS membrane. The tool uses a motor to suck all the air inside of the tool, creating a constant vacuum which in directly affects the bubbles inside of the formulation to come out. The method was tried for 120 minutes and a leak up test rate was calculated. The second method used was to bake the silicon wafers in the oven for 1 hour at 95°C. This method was the one who showed best results compared to the desiccator.

3.4.4. Etching Polymer time

The etch rate of the polymer was obtained by trial and error and has been determined to be 180nm per minute. This etch rate requires for the device to be continuously etched for 44 minutes. To optimize the etch time several batches were tested for different total etching time. In **Figure 3.36** and **Figure 3.37** there's evidence that the metal layer on the top is being damaged. The roughness of the surface and the darker colouring is suspected to be because the photoresist from the microporous patterning stage is already been depleted and the metal layer has no longer a masking layer.

The aluminium is not going to react with the fluorine plasma in the ICP etching. However, the ICP etching chamber is used with ion bombardment and radicals combined to have a better etching rate. The aluminium mask is not immune to the ion bombardment. When an ion impacts the aluminium surface layer, molecules from the metal layer will redeposit into the surface. This redeposition of material can cause micro masking. One example of this can be seen in **Figure 3.18**

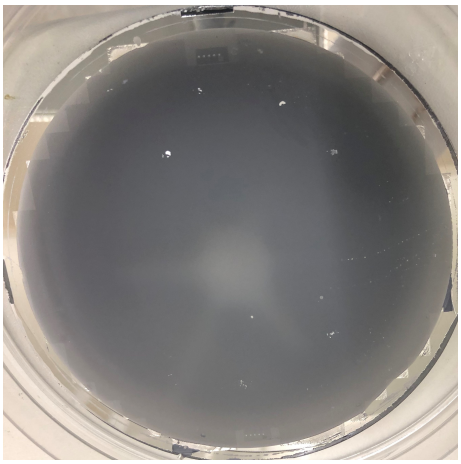

Figure 3.36: Polymer etching after 44 minutes

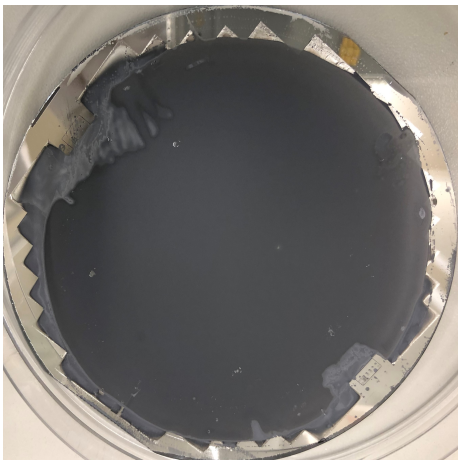

Figure 3.37: Polymer etching after 52 minutes

3.4.5. Broken membranes

In the step where the membrane is suspended a carrier wafer is used to protect the tools transport chuck and to provide a better surface for the tool to be able to hold the silicon wafer in place. During the removal of the carrier wafer the surface of the membrane suffers damage when the layers are detached. In **Figure 3.38** and **Figure 3.39** there are images of failed membranes. Out of 52 possible individual membranes the average of success per wafer is about 85% being 42 the lowest amount of membranes obtained.

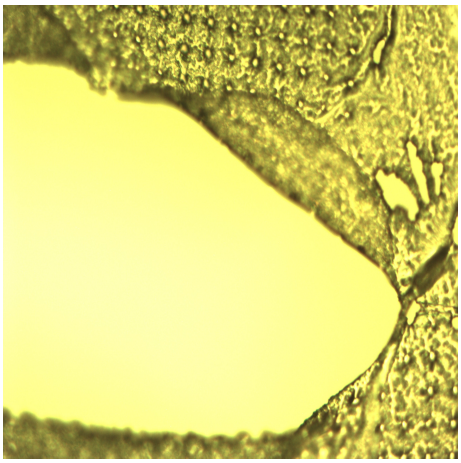

Figure 3.38: Broken membrane. Picture taken with optical microscope with 20X magnification

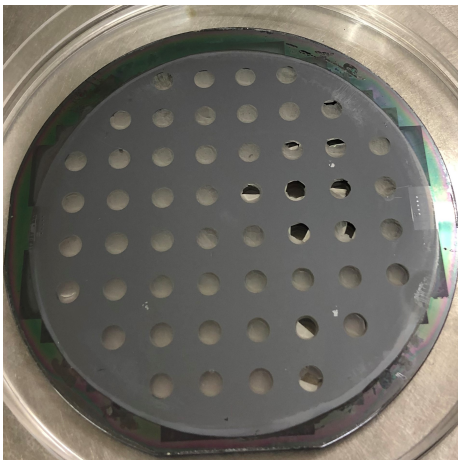

Figure 3.39: Broken membranes

3.4.6. Acid damage

Wet etching is required to remove the last oxide layer and the metal on top of the OSTE-PDMS. To do this, the use of acids such as BHF and PES remove the layers. Its important to note that these acids can attack the OSTE-PDMS layer and remove it from the device. One example of this can be seen in **Figure 3.40** and **Figure 3.41**, these two images help us compare how a successful membrane looks in comparison to an attacked membrane

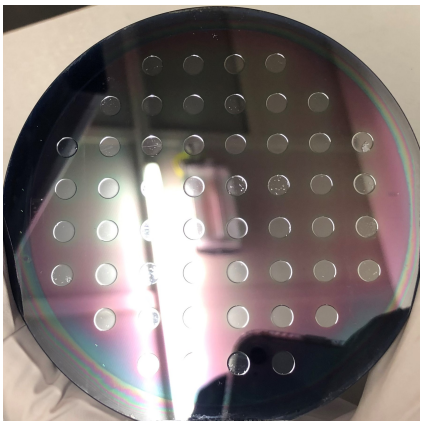

Figure 3.40: Suspended Unpatterned OSTE-PDMS membrane. Openings of 5mm in diameter separated by 1 cm

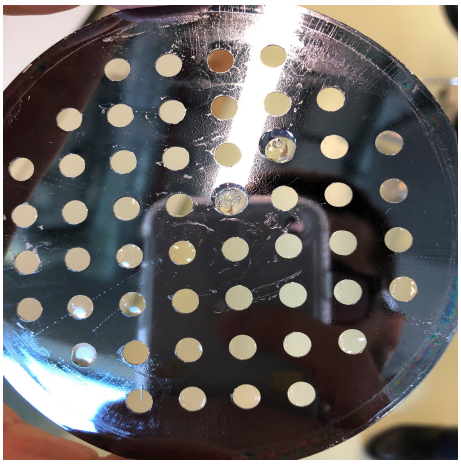

Figure 3.41: OSTE-PDMS Layer almost completely destroyed by acid attack

3.4.7. Dicing issues

The dicing tool is used to make precision cuts in the wafer to be able to have individual testing chips, each confirmed of an individual OSTE-PDMS membrane. One of the methods used during this process is to use a sticky foil mask in both sides of the wafer, to help keep the wafer together and for it to stay in place while the saw of the dicing tool cuts through the silicon wafer. In **Figure 3.42** the dicing process destroyed each of the membranes. The reason this happened was the foil adhered to the front side (the polymer side) and acetone, or isopropyl alcohol were not able to dissolve the glue. Every time the foil was separated from the chip the membrane was completely separated leaving an empty circle. To solve this several approaches can be made. The first is to test the fabrication with a stiffer formulation, the resulting membrane may be strong enough to be detached without suffering any damage. Another approach is to use photoresist on top of the OSTE-PDMS to provide some protection to the patterns in the dicing stage. Finally, another possible way is to dice the wafer before the final step of the removing of the metal layer, this method is believed to be the best since the foil paper was not attached to the back of the wafer.

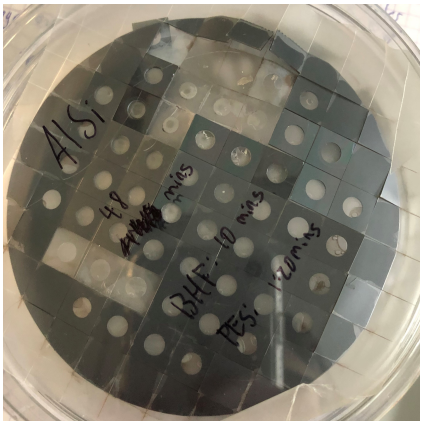

Figure 3.42: OSTE-PDMS membranes attached to foil

4

Hydrogel bonding

In recent years hydrogels research has been on the rise. [31] These materials are investigated and used in tissue engineering, therapeutics, regenerative medicine and also are used to create synthetic 3D networks for cell culturing. [28, 36]. Hydrogels posses a high volume of water content which helps it simulate soft tissue and mimic the environment of the cells [65]. Synthetic scaffold offer advantages such as being biodegradable, biocompatible and its mechanical and chemical characteristics can be customized [35, 61]. They consist of polymeric materials and water, used to mimic microenvironment physically and chemically [36]. Their formation involves the change of liquid precursors into solid by optical or chemical crosslinking.[7]. Mechanic characteristics such as stiffness, topography, elasticity and Young's Modulus are all variables that influence the cell. These properties contain critical information that need to be sensed by the mechanic receptor of the cells. [36, 61]

4.1. Poly Ethylene Glycol (PEG)

Polyethylene glycol (PEG) is a widely used synthetic hydrogel. Its formulation contains a mix of hydrophilic polymers that when chemically crosslinked have the capacity of holding water. This material has good properties such as custom viscosity and porosity.[36] The material is mostly used because it is one of the most hydrophilic materials and shows a low level of adsorption of proteins.[31]. Different functional groups can be attached to the end of polymer arms via some chemistry, due to this reason the use of the material has become more popular. [28]

The hydrogels tested during this project are based on two precursor polymers: Polyethylene glycol hydroxil (PEG-OH) and Polyethylene glycol thiol (PEG-SH). The PEG-OH is precursor to both Poly Ethylene Glycol Oxidized DiThiolene (PEG-ODT) and Poly Ethylene Glycol Oxidized DiThiolene (PEG-DT) the synthesis is done to obtain a chemical compound derived form acids called ester through a reaction called Steglich Esterification (DCC + DMAP). The reason for using this reaction is that the substrate is acid sensitive. Both of the resulting molecules are thiol reactive. The PEG-DT requires UV light to have a chemical reaction called ring polymerization where long chains of polymers are formed to form the network hydrogel. PEG-ODT needs to be mixed with PEG-SH to chemically crosslink and form the hydrogel instead of using UV light.

Freshly prepared formulations of OSTE-PDMS are preferred because the thiol groups in the surface can react and create new bonds called disulphide. [32] Once a bond of disulfide is made the functional groups are no longer viable as a focal point for thiol reactive molecules.

4.1.1. Poly Ethylene Glycol (PEG) DiThiolene (DT)

During the literature review most of the project data was based on the article by Liu [38] in the their project they used a UV cured hydrogel to bond with the OSTE-PDMS surface. During this project a similar approach was used. In **Figure 4.1** there's a diagram of the process to create the network. The molecule obtained has a functional group attached to the end of its 4 arms. The mechanism of PEG-DT to form a hydrogel is through a free radical initiated polymerization. The UV light will react with the photo initiator LAP to produce free radicals, these free radicals will induce a reaction in the four arm PEG to crosslink with each other. The radicals attack the S-S bond at the end of the chains to free thiolates. Through the crosslinking of the four

arm PEGs the hydrogel is formed. Once the network is created it will be thiol reactive and will bond with the thiol groups that the OSTE-PDMS has in excess in its surface.

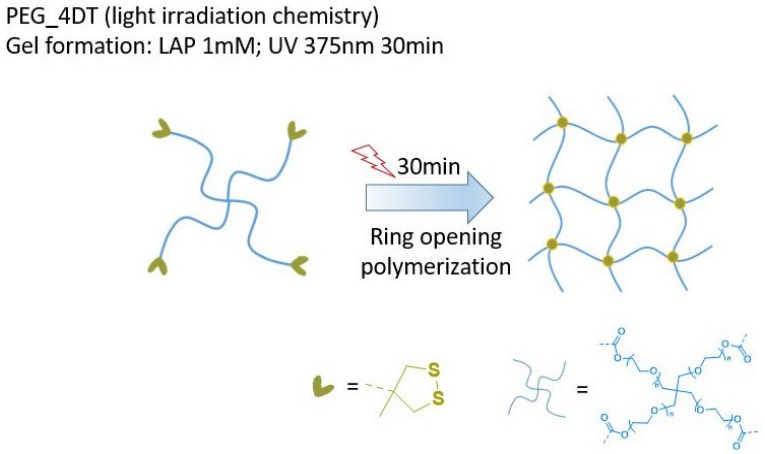

Figure 4.1: Diagram of the PEG DT

4.1.2. Materials and Methods

For the UV triggered hydrogel formation, a solution of PEG-DT (synthesized from PEG-4arm-OH) has to be dissolved in phosphate buffered saline (PBS). The photo initiator Lithium phenyl-2,4,6 trimethyl-benzoyl phosphinate (LAP) is also dissolved in PBS and mixed with PEG-DT solution. The solution is then exposed under UV (375nm) for 30 min for the hydrogel formation. The volume used is 2mL.

Materials:

1. PEG-4arm-DT (Synthesized from PEG-4arm-OH),
2. PBS,
3. Photo initiator Lithium phenyl-2,4,6 trimethyl-benzoyl phosphinate (LAP)
4. UV lamp (375nm, 10 mj/cm^2)

Test conditions:

1. PEG-DT (4mM),
2. UV (30min),

Method: Using **Figure 4.2** as reference, the method of implementation is to begin with a uncured OSTE-PDMS formulation inside a petri dish and cure it with UV light for 3s; then mix the PEG-DT and LAP in PBS solution, using a pipette pour it on the surface of semi-cured OSTE-PDMS, curing in UV light for 30 min.

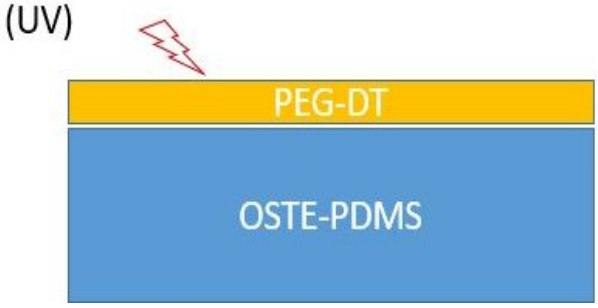

Figure 4.2: Test carried for the bonding of the hydrogel to the OSTE-PDMS surface

4.1.3. Results

During the testing of the hydrogel bonding two extra scenarios were tested. The use of a uncured OSTE-PDMS formulation to bond with the hydrogel and the opposite scenario where the formulation was cured before the pouring of the hydrogel. In **Figure 4.3** the results of a uncured formulation is shown, the reason for the hydrogel to concentrate is because the substrate wasn't providing a solid point for the hydrogel to be poured. In **Figure 4.4** the hydrogel was able to form on top of the precured membrane but the adhesion between the materials was weak and easy to break. Its important to mention that the yellow look of the images is due to the fact that the OSTE-PDMS formulation had a 2.5% solution of photo initiator inside.

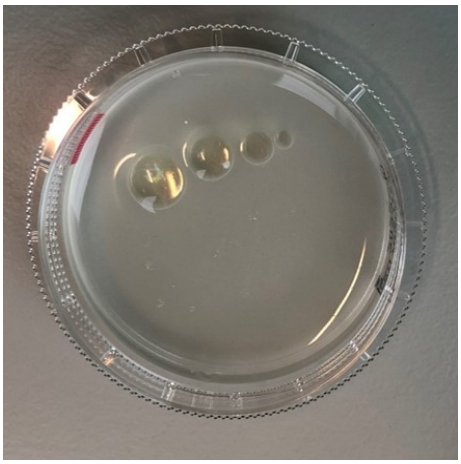

Figure 4.3: Uncured OSTE-PDMS and PEG DT sample

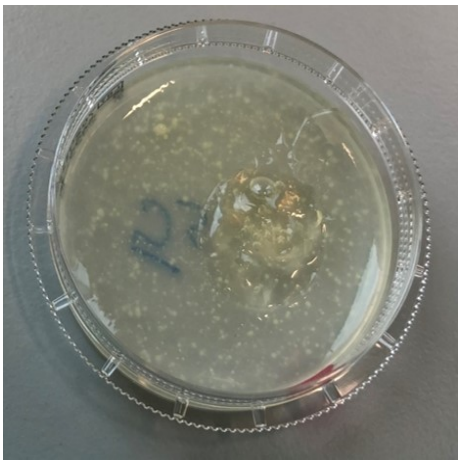

Figure 4.4: Cured OSTE-PDMS and PEG DT sample

In the **Figure 4.5** the OSTE-PDMS formulation was lowered to 0,5% of photoinitiator resulting in a optically transparent surface. The use of semicured OSTE-PDMS was to provide a solid surface while still having some unreacted material to bond with. The materials will be exposed together in UV light to cure together and provide a chemical bond were the adhesion is stronger.

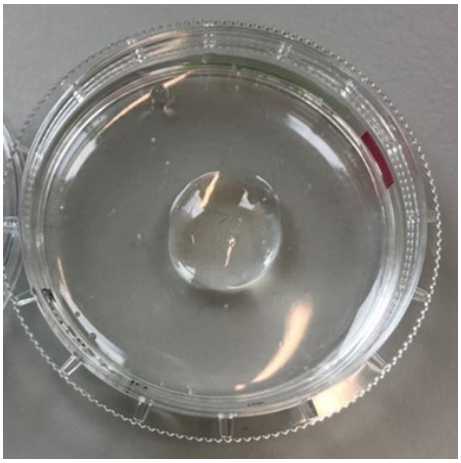

Figure 4.5: Semicured OSTE-PDMS and Cured PEG DT sample

4.1.4. Poly Ethylene Glycol (PEG) Oxidized DiThiolene (ODT)

The hydrogel purpose is to provide a microenvironment to the cells. Studies have shown that the presence of UV irradiation affects the cells.[65] The PEG-ODT follows the same procedure to form as PEG DT, for both molecules the precursor is PEG OH but to have a chemically induced reaction it needs to be mixed with PEG SH. Using **Figure 4.6** as reference these two polymers will react together to form the hydrogel network. This chemistry is initiated without the requirement of UV light exposure. The resulting network created will be thiol reactive and will bond with the thiol groups that the OSTE-PDMS has in excess in its surface.

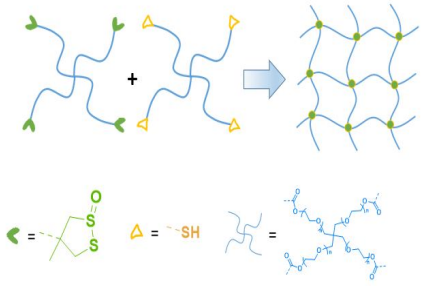

Figure 4.6: Diagram of the PEG ODT

4.1.5. Materials and Methods

The process for the gel formation is the following: use PBS, pH7.4, 10 mM. Then dissolve the PEGs (PEG-ODT and PEG-SH) separately in glass vials with 1 mM concentration. The gel formation is done by mixing the two precursor solutions and incubate at room temperature. Usually, for PEG-ODT and PEG-SH will react to form a hydrogel in an incubator in a temperature of 37°C in around 5min.

Materials:

1. PEG-4arm-ODT (Synthesized from PEG-4arm-OH),
2. PEG-4arm-SH
3. PBS

Test conditions:

1. PEG-DT (4mM),
2. PEG-4SH solution (6mM),
3. PBS

Method: Using **Figure 4.7** as reference, the method of implementation is to apply a cured OSTE-PDMS; prepare a PEG-4ODT solution (6mM) and a PEG-4SH solution (6mM) in PBS; mix the two precursors and apply on the surface of cured OSTE-PDMS, incubate for 5min;

Figure 4.7: Test carried for the bonding of the hydrogel to the OSTE-PDMS surface

4.1.6. Results

During this part of the experiment, the same testing was performed with a semi cured OSTE-PDMS formulation and with a cured OSTE-PDMS formulation. **Figure 4.8** and **Figure 4.9** both show to have a similar effect on the adhesion of the hydrogel to the OSTE-PDMS surface. In the pictures we can see the hydrogel, which is mostly made of water, to form a droplet on top of the hydrophobic OSTE-PDMS surface. The hydrogel in the opposite is a hydrophilic material. These antagonistic behaviours between the surfaces makes it impossible to spread the hydrogel on top of the surface without it creating drops. For this to be performed the OSTE-PDMS surface will need a treatment such as oxygen plasma to change its properties from hydrophobic to hydrophilic.

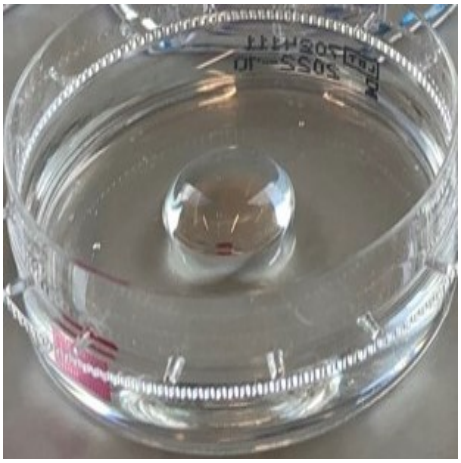

Figure 4.8: Semicured OSTE-PDMS and PEG ODT sample

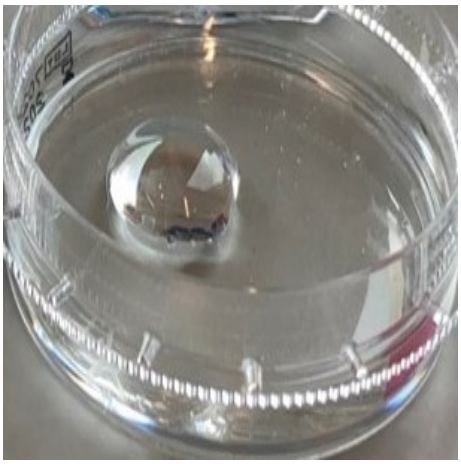

Figure 4.9: Cured OSTE-PDMS and PEG ODT sample

4.2. Integration to the OSTE PDMS Suspended Membrane

The final part of this project is to be able to have the integration of the hydrogel to the microporous suspended membranes fabricated in the previous chapter. Using **Figure 4.10** as reference the final device is exposed. The hydrogel is integrated to the surface of the OSTE-PDMS ensuring that the actuation of the membrane will be mechanically transferred to the hydrogel and finally to the cell culture. In **Figure 4.11** and **Figure 4.12** there are images where the PEG ODT was successfully integrated to the micro patterned membrane of OSTE-PDMS. During this stage the bonding between the surfaces was tested by placing the devices with the hydrogel integrated upside down to test if gravity could decouple the surfaces as well as testing with a pipette to try to push the hydrogel from its place.

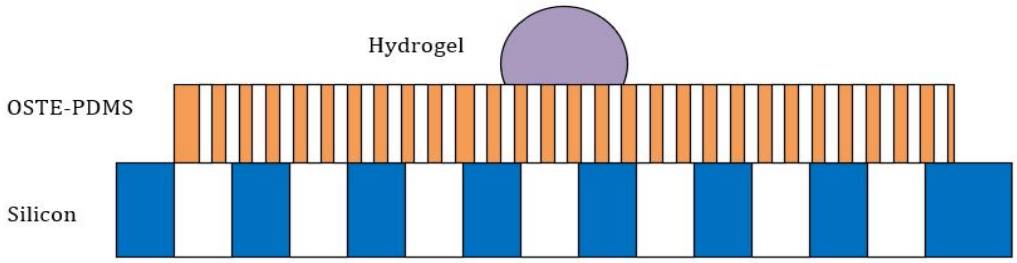

Figure 4.10: Cross section of the device

4.3. Discussion

4.3.1. UV Cured

The hydrogel was successfully integrated to a OSTE-PDMS membrane but the process to do it required a UV radiation for the chemical reaction to start and eventually form the hydrogel. This approach has two fundamental problems with the microporous implementation, the first being that studies have showed that UV radiation is not good for the cell culture [65] and the second one being that its not possible to micropattern a semi cured OSTE-PDMS membrane because is not compatible with the process.

4.3.2. Non-UV Cured

The hydrogel was successfully integrated into both the OSTE-PDMS semi cured and cured formulations. Both of the formulation showed similar results regarding the strength of their bonds. Due to similar results during this stage the method chosen to work with was the fully cured OSTE-PDMS due to the requirement of bonding the hydrogel to an already fabricated device.

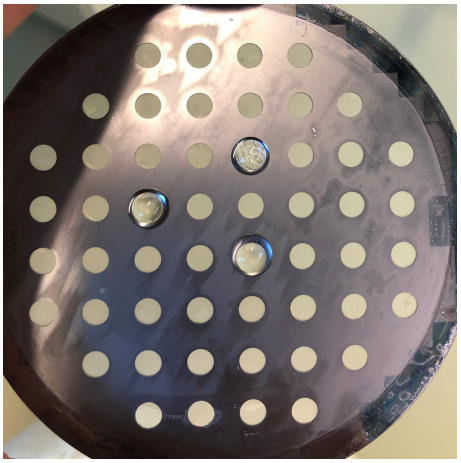

Figure 4.11: Suspended porous OSTE-PDMS and cured PEG ODT sample

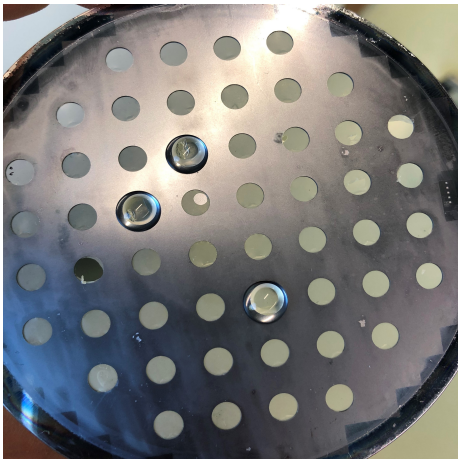

Figure 4.12: Suspended porous OSTE-PDMS and cured PEG ODT sample

4.3.3. Cell Viability

Cell culture tests need to be performed in the hydrogels to determine if cells are able to survive inside of the hydrogel and if its environment is biocompatible. The synthesis of the hydrogels used during the project is not the same as the one used by Liu [38] where it was used a arm hydrogel while in this project a 4 arm molecule was used. Additionally, a new form of chemically induced curing is used during the project to be able to avoid UV light irradiation to the cells and a faster curing time with an average of 10 minutes.

4.3.4. Dehydration of Hydrogels

Hydrogels are made of mostly water due to this the duration of a hydrogel before it collapses and dry itself out is about 48 hours. In **Figure 4.13** there's an example of a dehydrated hydrogel on top of PDMS. Due to this reason the studies need to be carried out require to be done very close to the bonding. Another possibility is to design an encapsulation for the hydrogel to isolate it from the atmosphere and adding excess water for the hydrogel to last longer.

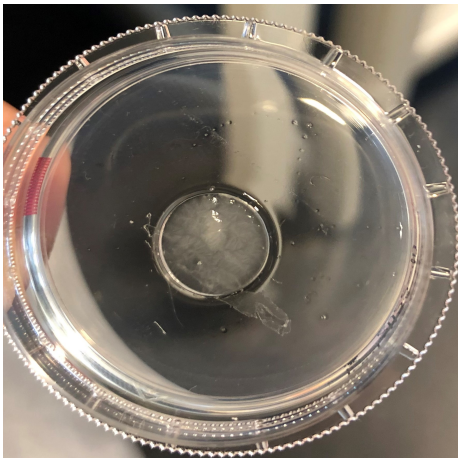

Figure 4.13: Dehydrated hydrogels

4.3.5. Hydrophobic surface

OSTE-PDMS has similar characteristics and it shares its hydrophobic behaviour. Due to the surface repelling the water its not possible to spread a hydrogel layer on top of the surface without them creating drop like shapes. To accomplish this, surface activation techniques can be used such as oxygen plasma allowing the potential to spread an even layer of hydrogel on top of OSTE-PDMS. This will make the fabrication of several OSTE-PDMS individual chips of a flexible microporous suspended membrane possible.

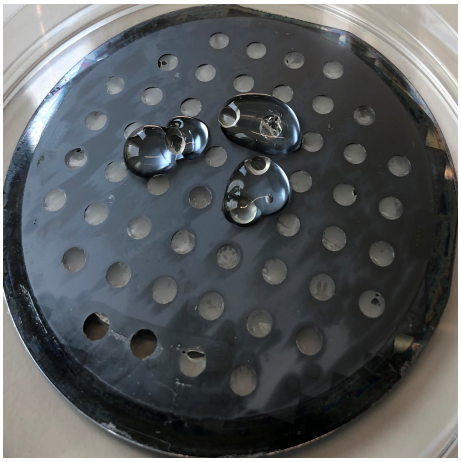

Figure 4.14: Water on the surface of the OSTE-PDMS

5
Conclusions

5.1. Research Objectives Achieved

The motivation for doing this thesis was to be able to provide an alternative material for OOC applications. In this project, we have successfully integrated a suspended microporous membrane to an ECM supporting membrane by providing a passive one step surface modification for other molecules to attach. This surface offers topology and functionalization to promote a better adhesion of the cells to the substrate. Well-like structures should be designed in order to provide an isolated space for the hydrogel with the culture, and to provide additional solution to keep the culture hydrated.

The device differentiates from literature by using fabrication techniques to obtain a pattern on top of the surface of the membrane. In the article by Liu [38] micromolding techniques are suggested to be able to pattern the surface, but these techniques make it difficult to pattern micropores into the surface. By using fabrication techniques, the microporous membrane is able to provide specific pores for selective signaling exchange through layers or cell cultures. Additionally, the curing process followed by Liu was via UV curing which requires a semicured layer of OSTE-PDMS. This is not compatible with the fabrication process.

In contrast, OoC fabrications that use PDMS have had to use some kind of surface activation or coating to be able to modify the surface of the interface. Compared to Liu, Quiros-Solano and Gaio [19, 38, 48], in this project a flexible microporous membrane was fabricated out of a material which doesn't require surface modification for it to attach ECM supporting membranes. This device is able to truly deliver a 3D stimulation to the cells inside of the hydrogel. The good adhesion between the layers allows for the cells to interact between each other and to sense the stimuli that the actuators are sending. Additionally, we used a new chemically induced hydrogel which avoids UV light and cures on average in 10 minutes. This gives it the ability to bond to a previously cured and fabricated custom surface.

The accomplishment of having the chemically induced hydrogel bond with a cured patterned OSTE-PDMS membrane is a great advancement as it can be used for different applications. During this project we proved that OSTE-PDMS can undergo similar fabrication process as regular PDMS. Taking advantage of its modified surface makes it a good material. Most of the already designed PDMS devices have to undergo some surface activation to be able to change its properties. Most of these techniques are not compatible with cell culture. The use of a cured OSTE-PDMS instead of a semi cured OSTE-PDMS gives the opportunity to design, fabricate and in latter stages allow the integration of an ECM supporting molecule. The polymers were characterized through tensile testing and dynamic tensile testing to undergo a mechanical characterization to reveal some of its properties. The devices have a elastic range of **0 to 140 kPa.**.The analysis of the loss modulus of OSTE-PDMS compared to the 20:1 PDMS (comparable Young's Modulus) revealed that this formulation may be too brittle to last a long time.

With the results of the project we have resolved our problem statement and have reached our research goals, but unfortunately there was no time for testing a cell culture in the hydrogel to assess its viability as a good ECM supporting membrane.

5.2. Recommendations and Future Work

The selection of OSTE-PDMS was mainly due to its rapid prototyping and its passive surface that can be modified to attach other molecules. The next stages of this work will have to focus on fixing:

- Deposition of an Aluminum layer on top of the OSTE-PDMS shows that stress is being generated during the process and wrinkles are created. To fix this, a stress buffering layer can be used to diminish the stress. This layer will be sandwiched between the OSTE-PDMS and the metal layer and will serve as an anchoring point. A flatter, smoother surface will result because of this, increasing the adhesion between the layers, avoiding issues such as delamination.
- The issue of degassing. The polymer and its surface should go through a process where temperatures above 37°C are avoided. If the leak up test required for the metal layer is not successful a different metal evaporation such as CHA Evaporator can be used.
- Different ratios of vinyl terminated to functionalized OSTE-PDMS should be tested to increase the stiffness of the material while still being able to bond a hydrogel.
- Material needs to start being tested in current applications that use surface modification techniques to increase adhesion due to the fact that some of these techniques are reversible and not always compatible with cell culturing.
- New material is able to transfer a 3D stimulation to the cell culture inside of the hydrogel. OoCs platforms that use cyclic stretching should try to use OSTE-PDMS as a material due to its surface capabilities.
- A smaller membrane should be spin coated to be able to have a polymer etching for a lesser time as the metal layer has shown evidence to being damaged by the ions during the polymer etching. Alternatively, a thicker photoresist can be used to protect the metal and polymers layers when the etching is performed.
- Dicing should be done in a way where the foil protecting the membranes can be placed to protect the process, but its adhesion point should not be with the surface of the device. An alternative method that can be tested is to perform the dicing with the metal layer still on top of the OSTE-PDMS.
- Testing of the success of a cell culture in PEG-ODT is yet to be done. Toxicity and bio compatibility in literature is possible but no research using these methods was found. Additionally, the dehydration of the hydrogel needs to addressed. It is believed that the cells will proliferate and generate their own ECM, but it is necessary to provide a longer window of studying than 48 hours. For this an encapsulation could be done where an excess of water is kept to maintain the hydrogel water or to diminish its rate. Oils can also be used to encapsulate the hydrogel avoiding exchange with the atmosphere.
- Testing regarding swelling of the material will need to be done since the literature states that OSTE-PDMS should be a suitable substitute to PDMS for addressing issues such as greater solvent compatibility, antibacterial effect [60]. Other tests such as gas porosity [25], low shrinkage stress and no sensitivity to oxygen. [8] also need to be proved.
- Hydrophobic behavior of the surface is still present, surface activation techniques such as plasma oxygen could be used to change surface to hydrophilic. Further investigation is necessary to see if surface activation will affect the functional groups in the surface.
- Once the dicing is achieved a device similar to the **Figure 2.5** in chapter proposed by Gaio [18] can be made with OSTE-PDMS to be able to test each suspended membrane with a hydrogel bonded on top. Actuators inside of the device are currently being studied to avoid the need of using a pump to provide the pressure.

A

Process Flows

A.1. Suspended Porous OSTE PDMS membranes for cell culturing

1. Start with 4-inch 500 μm DSP wafer.
2. Creation of Front Alignment Markers.
   (a) Spincoat 1.4 μm of SPR 3012, and perform a bake.
   (b) Expose the wafers on PAS 5500/80 automatic waferstepper. Use recipe "ZEFWAM" and set the exposure energy to 125 mj/cm^2. Mask: COMURK.
   (c) Develop the wafers on EVG 120. Use MF322 developer. Recipe: *Dev-SP*
   (d) Perform an inspection and check for the line width.
   (e) Plasma Etch the wafer using Trikon Omega 201 Plasma etcher. Run the recipe *URKNPD*
3. Cleaning the wafers
   (a) Use the Tepla plasma system to remove the photoresist in an oxygen plasma.
   (b) 10 minutes in 100% nitric acid.
   (c) Rinse in the Quick Dump Rinser with the standard program until the resistivity is 5 MΩ.
   (d) 10 minutes in boiling nitric acid (65%) at 110°C.
   (e) Rinse in the Quick Dump Rinser with the standard program until the resistivity is 5 MΩ.
   (f) Dry the wafers.
4. Creation of Back Alignment Markers.
   (a) Spincoat 1.4 μm of SPR 3012, and perform a bake.
   (b) Expose the wafers on PAS 5500/80 automatic waferstepper. Use recipe *ZEFWAM* and set the exposure energy to 125 mj/cm^2. Mask: COMURK.
   (c) Develop the wafers on EVG 120. Use MF320 developer. Recipe: *Dev-SP*
   (d) Perform an inspection and check for the line width.
   (e) Plasma Etch the wafer using Trikon Omega 201 Plasma etcher. Run the recipe URK_NPD.
5. Cleaning the wafers
   (a) Use the Tepla plasma system to remove the photoresist in an oxygen plasma.
   (b) 10 minutes in 100% nitric acid.
   (c) Rinse in the Quick Dump Rinser with the standard program until the resistivity is 5 MΩ.
   (d) 10 minutes in boiling nitric acid (65%) at 110°C.
   (e) Rinse in the Quick Dump Rinser with the standard program until the resistivity is 5 MΩ.

(f) Dry the wafers.

6. PECVD Oxide Deposition on the front

   (a) Oxidation: for 2000 nm of oxide, Use Novellus Concept One PECVD reactor at 400°C. Use group: undoped oxide and recipe *xxxsiostd*. Use the logbook for times

   (b) Measure the oxide thickness using Leitz MPV-SP.

7. PECVD Oxide Deposition on the back

   (a) Oxidation: for 5000 nm of oxide, Use Novellus Concept One PECVD reactor at 400°C. Use group: undoped oxide and recipe *xxxsiostd*. Use the logbook for times

   (b) Measure the oxide thickness using Leitz MPV-SP.

8. Creation of patterns for the suspended membrane in the back

   (a) Spincoat 3.1 μm of SPR 3012, and perform a bake.

   (b) Expose the wafers on Use EVG 420 Contact Aligner Tool. Box 399. Mask:4 energy to 245 mj/cm^2.

   (c) Develop the wafers on EVG 120. Use MF320 developer. Recipe: *Dev-SP*

   (d) Perform an inspection and check for the line width.

   (e) Etch the SiO_2 using Drytek Triode 384T. Run the recipe *stdoxide* for 12 minutes

   (f) Measure the oxide thickness using Leitz MPV-SP.

9. Cleaning the wafers

   (a) Use the Tepla plasma system to remove the photoresist in an oxygen plasma.

   (b) 10 minutes in 100% nitric acid.

   (c) Rinse in the Quick Dump Rinser with the standard program until the resistivity is 5 MΩ.

   (d) 10 minutes in boiling nitric acid (65%) at 110°C.

   (e) Rinse in the Quick Dump Rinser with the standard program until the resistivity is 5 MΩ.

   (f) Dry the wafers.

10. OSTE-PDMS Formulation

    (a) Use the weighing scale to mix the 6 components. The thiol-functionalized PDMS (SMS042) and Vinyl – Functionalized (VDT431) (by pipette) and Vinyl-Terminated PDMS of 1000 cSt (DMS V31), Vinyl-Terminated PDMS of 5000 cSt (DMS V35), Vinyl terminated PDMS of 10,000 cSt (DMS V41), (by pouring) in a ratio of 2:0.3:1.5:1.5:1.5 in a plastic disposable cup. Finally use the pipette to have 0.5%wt of Photo Initiator (TPO-L)

    (b) Use the Thinky Speed mixer for mixing and degassing. Program 1

11. OSTE-PDMS curing

    (a) Use the Lanz Reinraum Techniek manual coater. To achieve 8 μm of thickness. Use recipe *x OSTEPDMS 5u*.

    (b) Use cotton swabs to clean the front, edges and the back of the wafer with acetone. No PDMS residues are allowed in the back

    (c) Use the EVG 420 Contact Aligner tool to expose the OSTE-PDMS to UV light, for a time equivalent of 2100 mj/cm^2.

    (d) Bake with the Memmert Oven at 115 °C for 60 minutes

12. Metallization and Patterning

    (a) Leak Up Test Rate is required to know if the metal can be sputtered.

    (b) Use the *Trikon Sigma* sputter coater for the deposition of the masking layer Al or Al/Si layer on the process wafers. Use the recipe *Al-200nm-25C* or *AlSi-200nm-25C-PDMS*.

(c) Insert the wafers in the Manual HMDS for 10 minutes

(d) Coat the wafer with *SpeCo1.5-nlof-NoHMDS* 1.5 μm of nLOF 2020

(e) Expose it on EVG 420 for a time equivalent of 75 mj/cm^2. Use Box 481. Masks EC2060-V2 from slots 9-15

(f) Bake the wafers for 7 minutes in the Memmert Oven in 115°C.

(g) Develop the wafers with *SpeDev-NoPEB-noHB.* and inspect for the structures.

(h) Etch the metal layer on wafer using Trikon Omega 201 Plasma etcher. Run the recipe *Al03-25*

(i) Etch the polymer on the wafer using Trikon Omega 201 Plasma etcher. Run the recipe *PDMS4*for 44 minutes

13. DRI Etching of bulk Silicon

(a) Etch the wafers from the backside using *Omega i2L Rapier*.

(b) Use a Carrier Wafer

(c) inspections in between to check the rate of etching. Land on the oxide layer.

(d) Etch the SiO_2 on the front side using *Waferview*for 550 cycles

14. Wet Etching of SiO_2 and Metal Layer

(a) No Polymers allowed in wet bench, go to SAL Lab

(b) Wet etch the remaining oxide on back using BHF (1:7) for 10 minutes

(c) Clean the wafer with clean water for 5 minutes

(d) Wet etch the metal on the top using PES for 1,5 minutes

(e) clean the wafer with clean water for 5 minutes

(f) Dry the wafer

B

Curves

B.1. Stress vs Strain curves

B.1.1. PDMS 5:1

In this section we provide the curves obtained from the tensile testing. **Figure B1 and B2** The 5:1 sample was used as a reference point to compare to OSTE-PDMS as they both have a 5:1 ratio for its formulation.

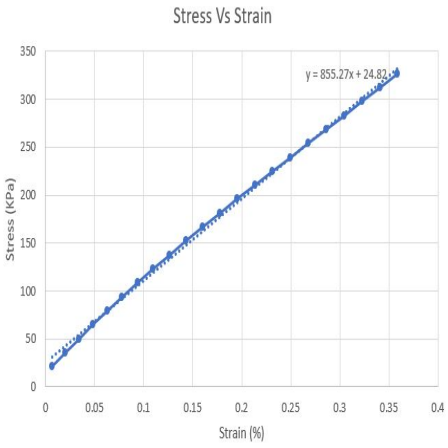

Figure B.1: Stress vs Strain curve PDMS 5:1

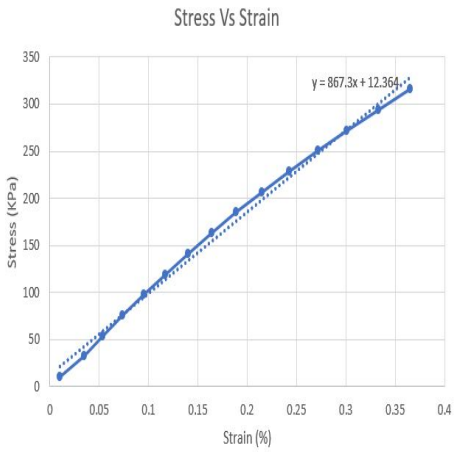

Figure B.2: Stress vs Strain curve PDMS 5:1

B.1.2. PDMS 10:1

In this section we provide the curves obtained from the tensile testing. This sample is the most widely used ratio 10:1 in PDMS, therefore it was used as control point to compare to the OSTE-PDMS formulation **Figure B3 and B4**

B.1.3. PDMS 20:1

In this section we provide the curves obtained from the tensile testing. **Figure B5 and B6** The 20:1 sample was used as a reference point to compare to OSTE-PDMS as they both have a similar Young's modulus

B.1.4. OSTE-PDMS control

In this section we provide the curves obtained from the tensile testing. **Figure B7 and B8** The ratio used during this test was 2:0.3:1.5:1.5:1.5. The same ratio was used by Liu [38] because it was solid enough yet flexible enough to create a membrane. Additionally it has a high amount of reactive thiol functional groups to bond with

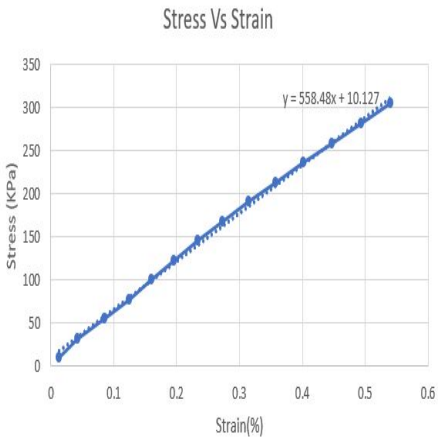

Figure B.3: Stress vs Strain curve PDMS 10:1

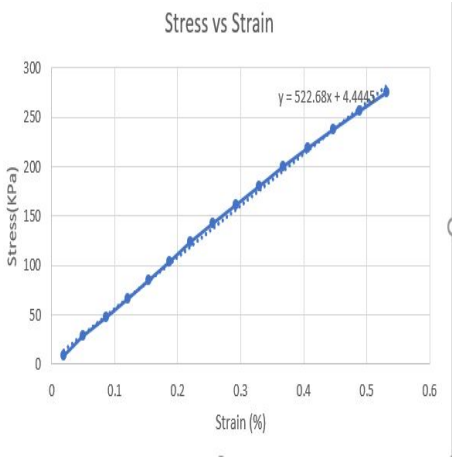

Figure B.4: Stress vs Strain curve PDMS 10:1

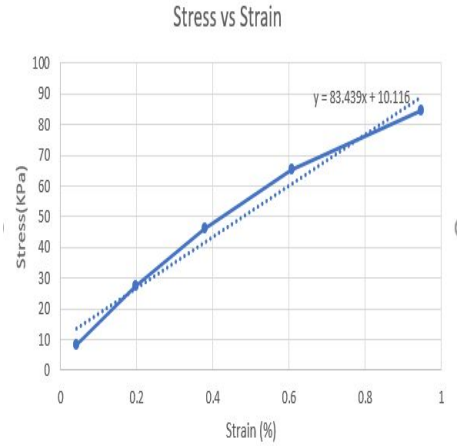

Figure B.5: Stress vs Strain curve PDMS 20:1

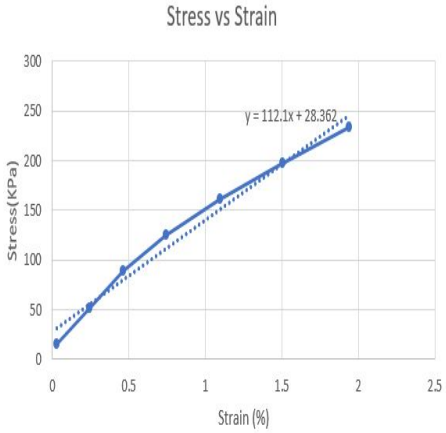

Figure B.6: Stress vs Strain curve PDMS 20:1

Figure B.7: Stress vs Strain curve OSTE-PDMS

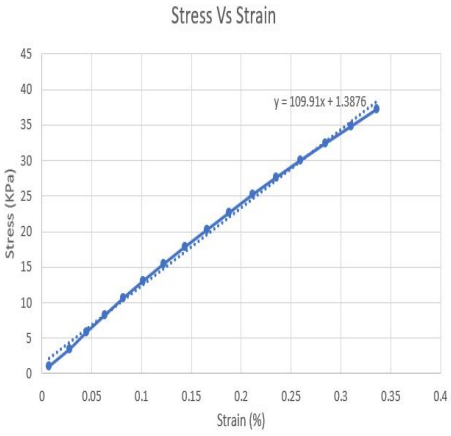

Figure B.8: Stress vs Strain curve OSTE-PDMS

B.1.5. OSTE-PDMS and hydrogel DT

In this section we provide the curves obtained from the tensile testing. The ratio used during this test was 2:0.3:1.5:1.5:1.5. The sample was obtained by doing a UV chemistry crosslinking of both the polymer and hydrogel. The hydrogel is physically bond to the surface by its thiol reactive functional groups in its end which is attracted to the surface of the OSTE-PDMS.

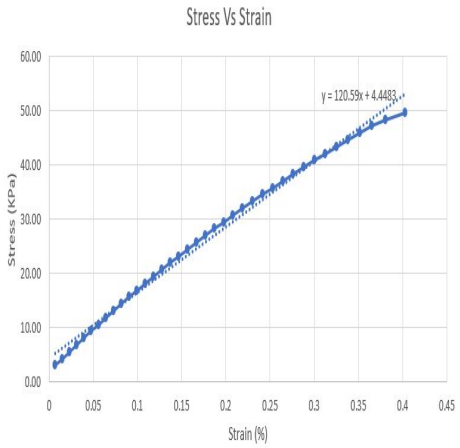

Figure B.9: Stress vs Strain curve OSTE-PDMS and PEG4DT

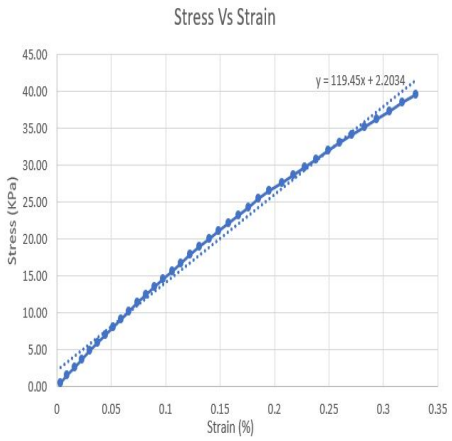

Figure B.10: Stress vs Strain curve OSTE-PDMS and PEG4DT

B.1.6. OSTE-PDMS and hydrogel ODT

In this section we provide the curves obtained from the tensile testing. The ratio used during this test was 2:0.3:1.5:1.5:1.5. The sample was obtained by doing a chemical induced chemistry crosslinking of both the polymer and hydrogel was made at 37C. The hydrogel is physically bond to the surface by its thiol reactive functional groups in its end which is attracted to the surface of the OSTE-PDMS. This is the highest Youngs modulus of all the OSTE-PDMS samples in this project.

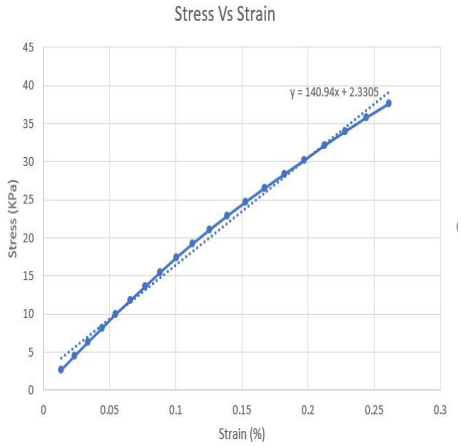

Figure B.11: Stress vs Strain curve OSTE-PDMS and PEG4ODT

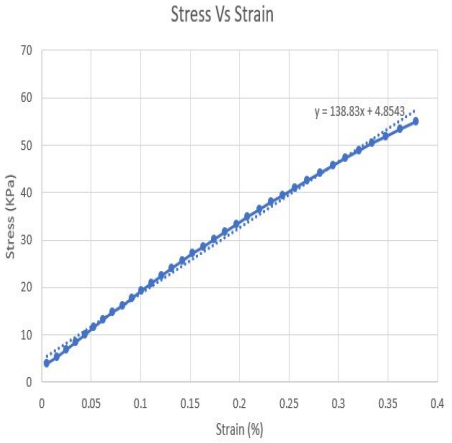

Figure B.12: Stress vs Strain curve OSTE-PDMS and PEG4ODT

B.2. Coating curves OSTE-PDMS

During this stage several samples were spin coated at different speeds and different times to achieve a thin and very even flat surface with a known profile. This is particularly important as the thickness of certain layers may affect some parts of the process.

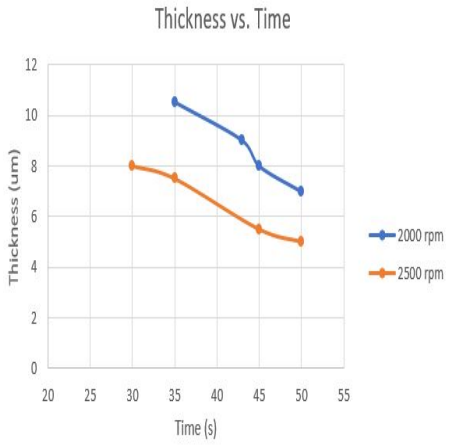

Figure B.13: Thickness vs Time

C

List of Acronyms

Acronym	**Meaning**
ECM	Extra cellular matrix
PDMS	Polydimethylsiloxane
OSTE-PDMS	Off-stoichiometry-thiol-ene polydimethylsiloxane
PEG	Polyethylene glycol
PEG OH	Polyethylene glycol hydroxil
PEG SH	Polyethylene glycol thiol
PEG DT	Polyethylene glycol dithiolene
PEG ODT	Polyethylene glycol oxidized dithiolene
PBS	Phosphate buffered saline
SiO_2	Silicon Oxide
BHF	Buffered oxide etch
Si	Silicon
PES	Phosphoric acid - Acetic acid - Nitric acid mixture
SEM	Scan Electron Microscope
OoC	Organ-on-chip
DRIE	Deep Reactive Ion Etch
PECVD	Plasma Enhanced Chemical Vapor Depositon
UV	Ultra Violet
PVD	Physical Vapor Deposition
ICP	Inductively Coupled Plasma Etching

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