AMERICAN UNIVERSITY OF BEIRUT

PROTOTYPE DEVELOPMENT OF A NOVEL BIO-MIMETIC LAB-ON-CHIP FOR STUDYING CANCER METASTASIS IN THE BREAST DUCTAL SYSTEM

by
WADDAH ARKAN MALAEB

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WADDAH ARKAN MALAEB

Approved by:

Dr. Ramsey Hamade, Professor — Advisor
Department of Mechanical Engineering

Dr. Rami Mhanna, Assistant Professor — Co-advisor
Biomedical Engineering Program

Dr. Rabih Talhouk, Professor — Member of Committee
Department of Biology

Dr. Kamel Ghali, Professor — Member of Committee
Department of Mechanical Engineering

Date of thesis defense: September 10, 2018

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AN ABSTRACT OF THE THESIS OF

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Title: Prototype Development of a Novel Bio-Mimetic Lab-On-Chip for Studying Cancer Metastasis in the Breast Ductal System

Breast cancer is the most common cancer and the second leading cause of mortality among women after lung cancer. Lab-on-a-chip (LOC) technology is shown to provide a structurally and functionally biomimetic environment that could be easily visualized in real-time. In this project, we designed a novel LOC model, which to our knowledge, is the first to mimic the full 3D ductal system of the human breast, with bio-mimetic structures, tissue types and matrix properties. Our system is composed of a thin porous circular cross-sectional duct, with the capability to assemble the proper extra cellular matrix (ECM) basal membrane, in addition to both the ductal epithelial tissue and the fibrous one on both sides of the duct.
This work was carried out through performing several prototyping stages on the LOC model and testing it mechanically and biologically. Initially, several solutions were proposed, and a conceptual design was selected according to a set of needs and specifications. Then, an embodiment design was articulated through many modifications and experimental verification of the selected materials and design parameters. Finally, the detailed design was created after optimizing the manufacturing and assembling process through a large set of experimental iterations. The final design was then re-fabricated, tested mechanistically for leakage & precision, and assessed biologically for cellular viability & attachment. The next step will be optimizing the cellular ductal buildup and the experimental conditions for cancer migration testing.
As a conclusion, this chip can be utilized by cancer researchers to study cancer invasion (extravasation & intravasation), metastasis, proliferation and even differentiation. It is designed in a way that can help investigate the prebuilt cellular structure for any specific physical, mechanical or molecular constituents inside and outside the duct in the fibrous tissue. Interestingly, our chip can be used on ductal systems other than breast such as lung, kidneys, glands, pancreas and heart as it can be easily manipulated to form different duct diameter sizes. In addition, this chip can also be used by pharmaceutical companies for high throughput testing on drugs' efficacy, delivery and targeting in ductal systems.

Key words:
Lab-on-a-Chip, Breast Cancer, Metastasis, Ductal System, Micro-Fluidics, Polysaccharides, GAGs, Basal membrane, Micro-manufacturing, Thermoplastics, PDMS, MDA-MB-231 cells, HMT-2522 S1 cells, Collagen I, Matrigel.

CONTENTS

ABBREVIATIONS

LOC	Lab-on-a-chip
MEMS	Micro Electro Mechanical Systems
CNC	computer numerical control
HUVEC	Human umbilical vein endothelial cells
IGF	Insulin-like growth factor
UV	Ultraviolet
HEC 293	Human embryonic kidney cells 293 Cells
PC	Polycarbonate
PET	Polyethylene tetraphalate
PMMA	Poly (methyl methacrylate)
PDMS	Poly di-methyl sulfone
PEEK	Polyether ether ketone
PTFE	polytetrafluoroethylene
Tg	Glass transition temperature
Tm	Melting temperature
ECM	Extracellular matrix
BM	Basal membrane
IDC	Invasive ductal carcinomas
DCIS	Ductal carcinomas in situ
VEGF	vascular endothelial growth factors

FIGURES

TABLES

CHAPTER I.

INTRODUCTION

A. Introduction to the Biology of Breast Cancer Metastasis and Methods of Experimentation

1. *Introduction to the Biology of Cancer Metastasis*

a. Breast Cancer Metastasis

While significant findings had been recently sought in the field of cancer research, mainly through the advancement of the fields of genomics, proteomics and molecular biology, the metastasis of cancer is still not well defined while being the cause of more than 90% of cancer deaths [1]. Breast cancer is still the most common cancer type and the second most common cause of cancer death in women after lung cancer [2], and invasive ductal carcinoma is the most frequent type of breast cancer (50-80%) [1]. Most of breast cancers emerge from the inner layer of luminal and epithelial cells at the end of mammary ducts connected to the lobules, this creates the need to study, model and visualize the mechanisms and causes by which cancer migrates from this site and metastasize through intravasation elsewhere in the body [3].

b. Biology of Breast Ductal Carcinomas

There are around 8 different types of breast cancers, that emerge from either the ducts, the lobules or the area in between, while the most common types are the ones that emerge in the ducts[4]. There are two main types of ductal carcinomas, invasive ductal carcinomas (IDC), and ductal carcinoma in situ (DCIS). DCIS is the most common type of non-invasive breast cancer, DCIS emerges in the mammary ducts and remains localized, doesn’t spread, but usually

increases the risk (30%) of developing an invasive breast cancer at a later stage [5]. On the other hand, about 80% of all breast cancers are IDC, which is the most common and deadliest type of breast cancers. IDC begins in the mammary ducts, but it tends to metastasize to the lymph glands through the lymphatic nodes or to other parts of the body through blood vessels [6]. IDC starts forming on the inside of the ducts, but it quickly tends to break the basement membrane of the duct, and then it migrates to the fibrous tissue surrounding the duct and starts to colonize there. Afterwards, cancer cells create a medium that induces angiogenesis by secreting a larger than normal amount of vascular endothelial growth factors (VEGF), increasing the number of blood vessels in the tissue, thus easing the process of intravasation into the vessel through angiogenesis [7]. The cells that enter blood vessels are then transported to another organ/tissue through the circulation [8].

c. In Vitro and In Vivo Models

In vitro 2D cultures had helped throughout the years in shaping the basis of cell biology through its simplicity, ease of handling and the capability to easily find the basic properties of each cell line and the effect of various drugs on them, in terms of cytotoxicity, viability, and proliferation [9]. **But 2D cultures suffers from many limitations** mainly because in 2D culture the cellular morphology changes, leading to a higher proliferation rates and a de-differentiation. Hence, it is a highly non-biomimetic cellular environment [10]. As an outcome, **3D culture rises to fame,** especially after the advancement in the hydrogel technology, where hydrogels became more like the ECM, and thus act as the native environment for the cells to grow on, inducing more natural cellular interaction between cells and with their environment, in addition to a better viability, proliferation and differentiation control [11]. In vivo models are the best; the tests are 100%

representative and give more confident results. But, **in vivo experiments suffers from many limitations** mainly the most prominent is that we have no controls of all the pathways and probabilities (many parameters interfere), and that we cannot clearly observe/detect/measure the effects resulting from a certain experiment [12]. Thus, researchers recently moved onto **creating a micro-manipulated complex 3D** in vitro models that best mimic the in vivo environment, namely, **the lab-on-chip (LOC) technology** [13].

d. Advanced Culture Models (3D printed models)

The advancement in the 3D bio-printing applications had paved the way to create different tissues and models with various shapes and sizes [14]. For an instance, the most recent work on 3D printed breast duct models is the work done by Ethan and Sokol at MIT, which is published in the Breast Cancer Research journal in March 2016. They seeded primary human breast epithelial cells into 3D hydrogels composed of transparent ECM proteins and carbohydrates (can be visualized under the microscope), those cells rapidly self-organized in the absence of stromal cells, and then within 2 weeks, formed mature mammary tissues with the correct cell topological orientation (polarity) and tissue morphologies (ducts & lobules), in addition that the ducts branched at the location where clusters expressed putative stem cell markers, they also controlled the expanding duct hollowness by treating with estrogen and progesterone hormones [15]. The aim of their work was to study the growth, development and biology of the mammary gland which proved to be successful using 3D printing application, but when we aim to study the effect of a drug, or that of cancer and metastasis on breast or other ductal tissues, we need the structural parameters to be controlled, since we need the accessibility to the inner walls of the duct after the maturation of the duct. This seems not to be the case in current 3D printing applications, because

of the uncontrollability of the duct location, size formation and branching, simply because the mechanisms by which those parameters are controlled aren't yet understood, knowing that the science of tissue engineering through current 3D printing applications are helping in building the basic knowledge on this issue [16].

B. Lab-on-a-Chip (LOC) Technology

1. *LOC Technology Development*

The advancement in the microfluidics field combined with the need for automated real-time measurement of the biological environment, using a minimal amount of materials and energy supply, gave rise to the idea of creating micro-biomimetic environments on microchips, that are more representative, and can be studied with a lower cost [17]. The advancement in the Micro-Electro-Mechanical Systems (MEMS) fabrication systems usually used for electronics and instrumentation manufacturing field, made this idea a reality. Lithography fabrication systems had shown to be able to create structures with features in the range of the nano-meters, these systems are commonly used in creating current lab-on-chip designs and is mostly used at the industrial level of manufacturing current LOC devices. For example, chemotaxis and migration assays had varied widely over the past decades. It began with the Boyden Chamber, known with the 2D transwell membrane assay, but because in the Boyden chamber we cannot visualize the migration path, a Zigmond chamber was developed in the late 1970s, where migration could be visualized and imaged across on a coverslip across a narrow constriction (10s of um). Afterwards the Dunn and install chamber was developed, which have improved high resolution log-term imaging competences for visual migration assays (Fig. 1) [18].

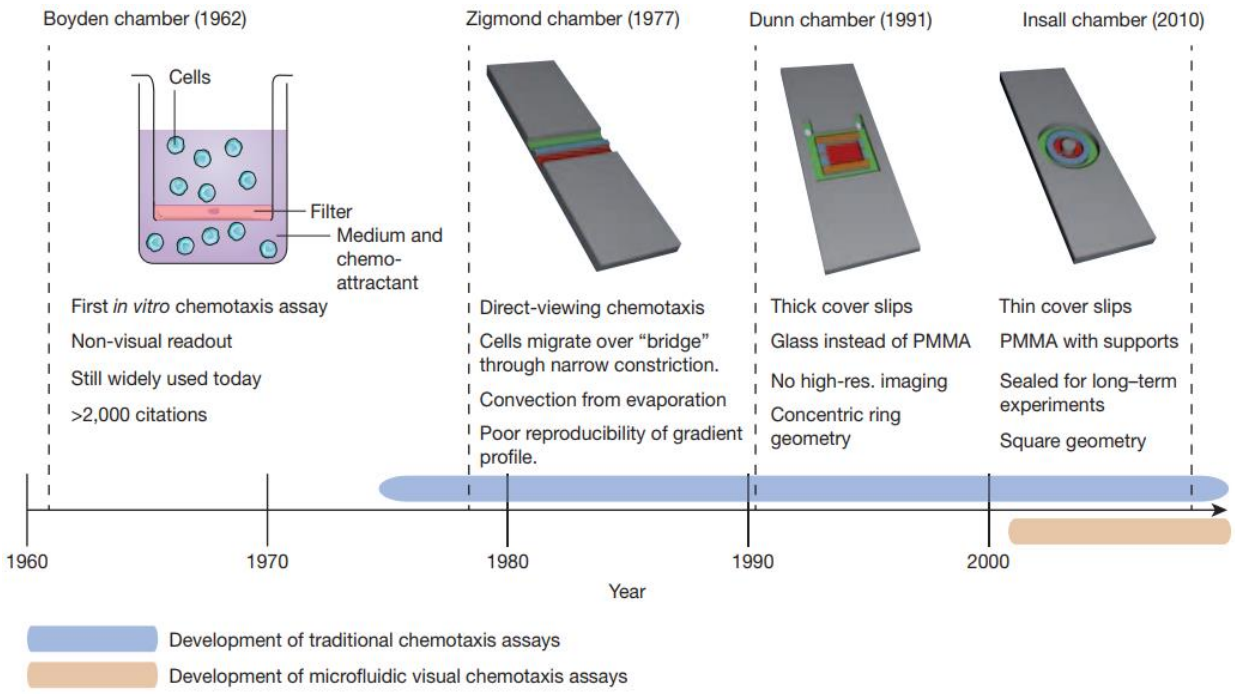


Figure 1. The development of visual chemotaxis assays over time (1960-present) [18].

2. *Current Advancements in LOC Devices*

Current LOC designs vary from cheap but basic microfluidic system, to costly and more advanced systems that are still on its verge of advancement. Various successes had been shown in the design of various organs/ organoids/tissues on chips that mimics the spleen, lungs, nerves, endothelium, skeletal muscles, bone marrow-liver tumor, cardiac network, vessels and intestines, all with the very specific structural and functional details [19] (Fig. 2a-2j). For instance, the functionality of breathing in lungs is mimicked by the seeding of 2 different cells, epithelial and vascular on the two sides of a stretchable membrane, the first is subjected to air, and the other to blood, this perfectly mimics the functional micro-environment of the lung cells, but still not the structural environment, since the membrane in this case is flat unlike in vivo.

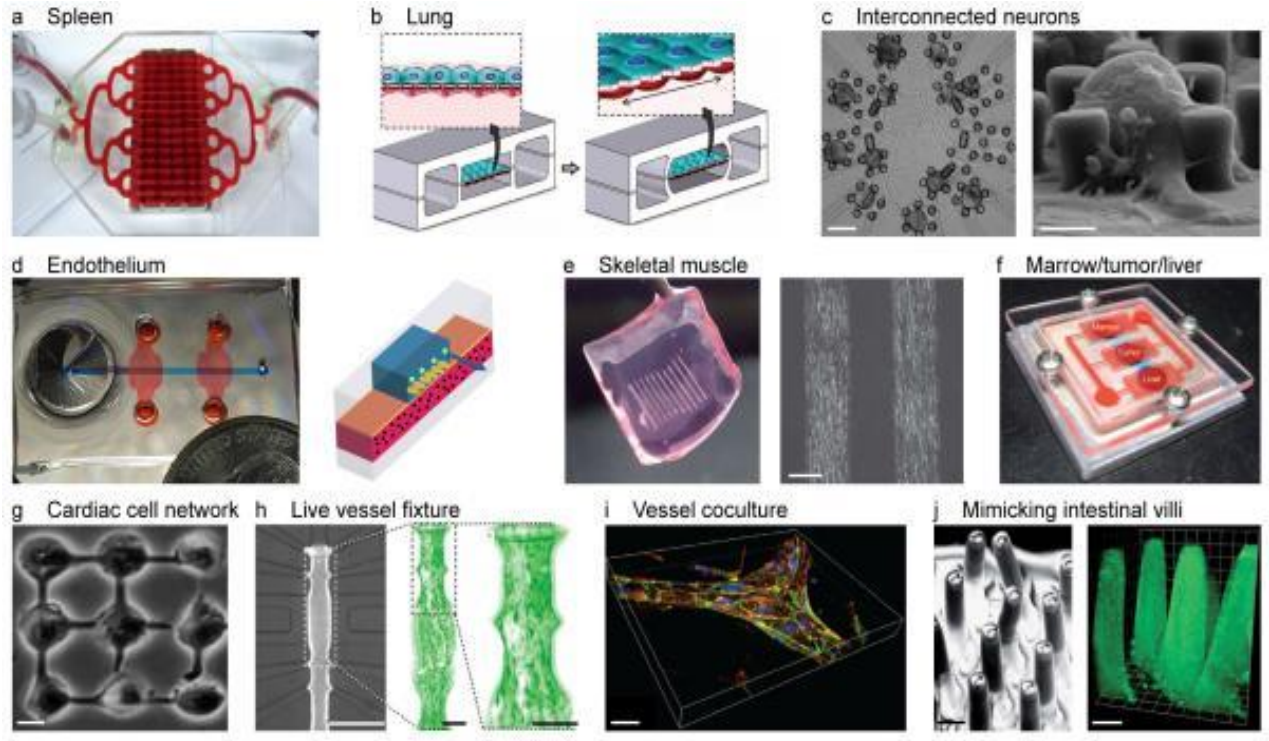


Figure 2. Microscale on-chip tissue models and culture platforms [19]. *LOCs of the (a) spleen, (b) lungs, (c) nerves, (d) endothelium, (e) skeletal muscles, (f) bone marrow-liver tumor, (g) cardiac network, (h & i) vessels and (J) intestinal cells.*

Another LOC example is that for breast on chip systems, and breast cancer metastasis models, the most prominent systems are the ones that either mimics the structure of the breast duct, or the ones that mimics the functional parameters of migration across to different tissues, still no system till today have both properties. One of the most well-known systems is the one developed by Sophie Lelievre's team at the university of Perdue, it is based on a hemi-channel of different material stiffness's and diameters, and aims to study the effect of the channel geometry on the arrangement of the cells and on mimicking and studying the tumor growth in those ducts (Fig. 3)[20]. **A major limitation for this system, compared to our proposed model, is the lack of a complete ductal structure (instead a hemi-duct is assembled).**

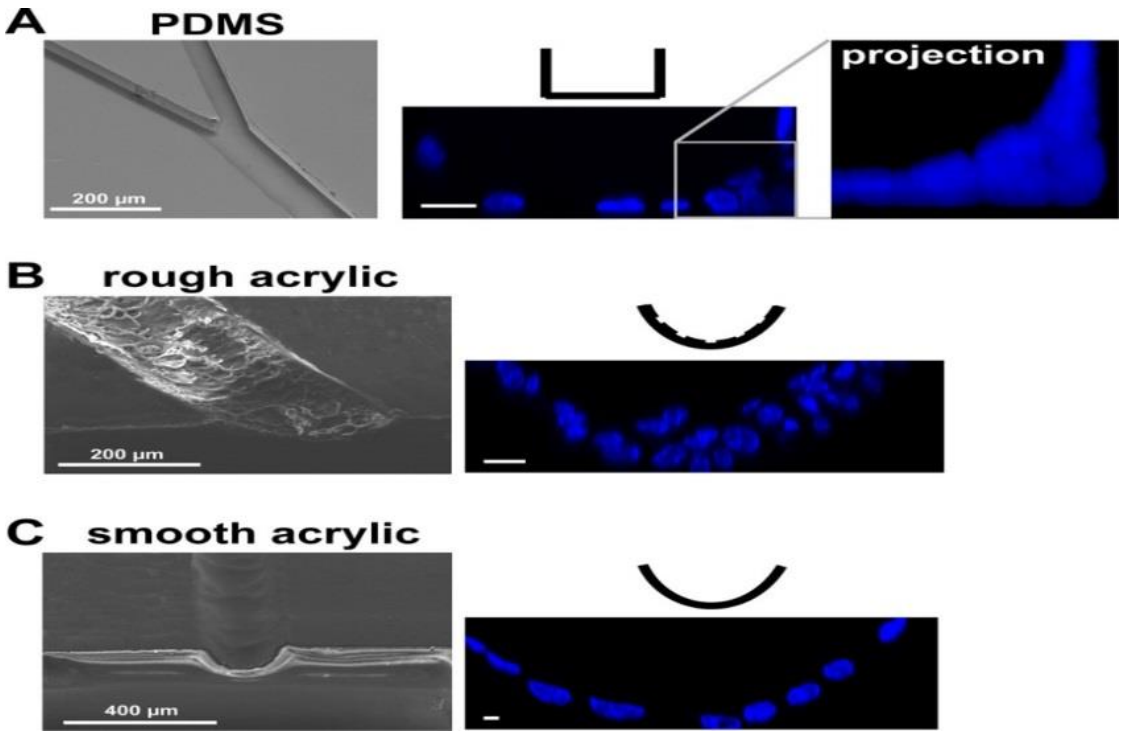


Figure 3. Hemi-Duct designed by Sophie Lelievre's laboratory at University of Purdue. [20]

Another highly advanced LOC cancer metastasis design is the one built by Roger Kamm's team at MIT (Fig. 4), in this system the aim was to study the cancer metastasis microvasculature visualization for the quantification of tumor cell extravasation dynamics. They built a chip as shown in Figure 4 below, it contains three channels that could be filled with cells, and 4 channels for media passage, that are in contact with both sides of all the cell filled channels. The two channels at the boundaries are filled with fibrous cells of the lungs (FB), suspended in fibrin gels, and the one at the middle is filled with human umbilical vein endothelial cells (HUVEC). Tumor cells or metastasis inducing molecules are passed in one of the media channels between the HUVEC and the FB, and the micro-vessel bed formation is visualized, where the vascular cells expanded from the middle channel and interconnected throughout the cross section of the media channel [21]. A similar system developed by the same team was used to study the 3D

vascularization and metastasis of breast cancer [22]. **However, a limitation of this system, compared to our proposed model, is the inability of the cells to assemble into open-ended ductular-like structures (instead spheroid cellular aggregates self-assemble) that allows streaming of fluidics within the through the ducts coated with apically polarized cells.**

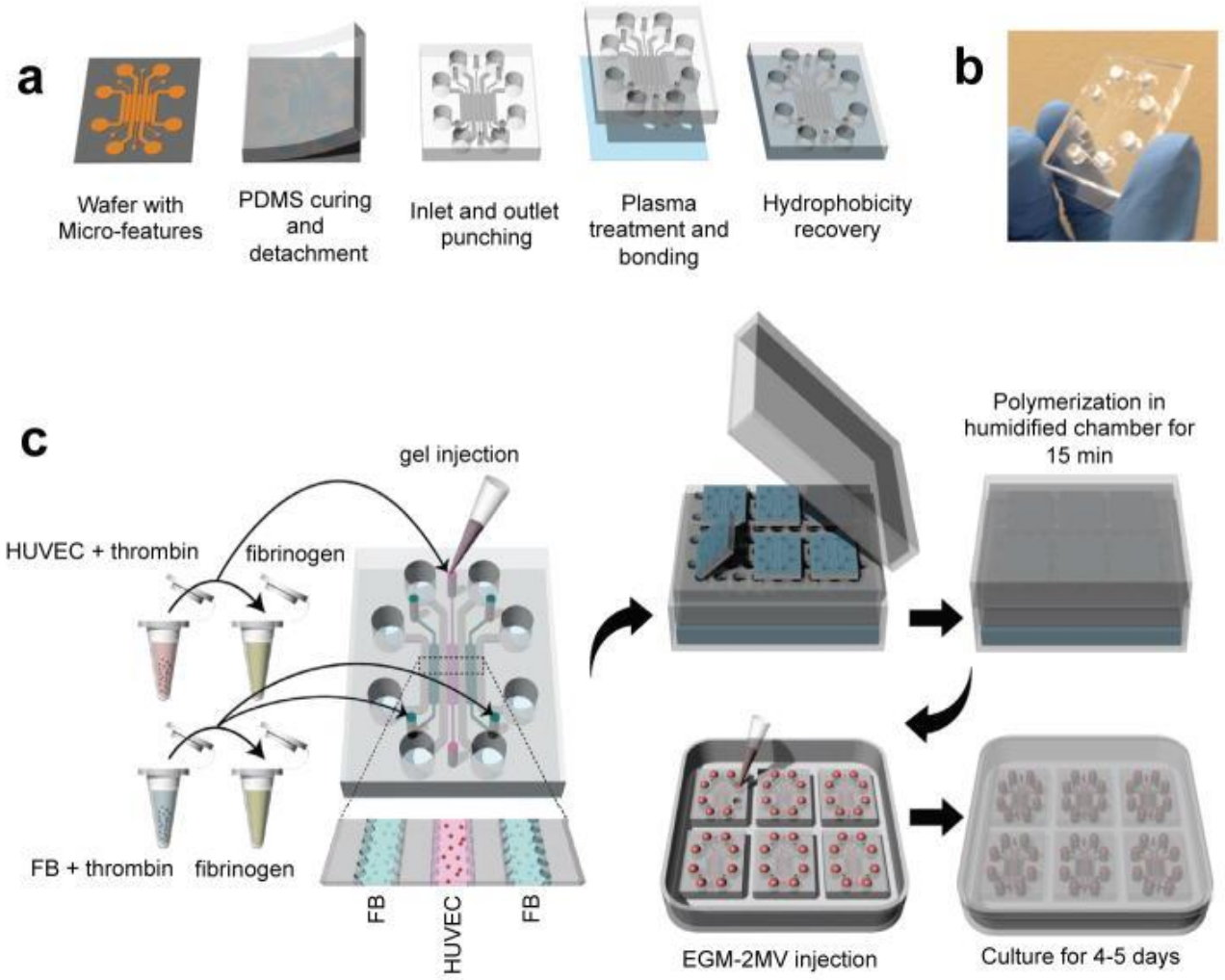


Figure 4. Roger Kamm's LOC design at MIT, schematic of design fabrication and cell seeding protocol [21].

CHAPTER II.
LOC DESIGN PROCESS

The LOC design process chosen is an iterative process based on five main design stages, ensuring the development of the optimal solution to the defined problem. The process goes by developing the optimal design, selecting & modifying materials to use, selecting fabrication process, treating & enhancing surfaces, and finally testing the design mechanistically and biologically.

The first stage is to identify the problem needed to be solved, through setting all the needs and requirements based on the scientific knowledge and bio-mimicry to the real tissue features and environment. Then, classify precisely all the needs and specifications, from the most essential aspect to the furthermost minor aspect needed, based on the scientific logic, the future perspectives from the literature, and the technical needs of the industry and the experimental research fields.

The second stage was to develop a conceptual design through an iterative loop that begin by proposing all the possible solutions to the problem based on current designs in the microfluidics field and further useful designs in other related fields. Then to select a conceptual design, each of the designs should be selected, analyzed and compared with all the other set designs, and then enhanced and modified to fit all the set needs and specifications. If the enhanced chosen design fits all the needs and specification, progress to the embodiment design stage, else select another design, analyze, compare, enhance and then iterate until the optimal design is found.

The third stage was to develop, modify and optimize the embodiment design by choosing the optimal materials to use for the design, based on the set design needs and specifications. And then optimize the geometry and dimensions configurations for each of the detailed aspects of the design based on the resemblance to the real tissue, fabrication ability, microscopic visualization ability, ease in assembling and connecting to other parts of the design and being user friendly when handled. Afterwards, for the chosen materials, the mechanical, chemical, optical and cost limits should be determined through market/literature, analytical, computational or experimental data. And finally, the materials should be tested biologically, optically and mechanically to determine if it fits in the set needs and specifications. If the chosen materials fit all the needs and specifications, then progress to the detailed design, else choose another material, optimize the geometry accordingly, determine the design limits, then test, modify and iterate until an optimal solution is found.

The fourth stage is the detailed design processes selection, optimization and testing. This stage is also based on iteration; one should first choose the manufacturing process and the assembling process of the detailed design chosen materials, based on the knowledge from the literature and industry, and on the available facilities. Afterwards, optimize the manufacturing processes and cutting parameters through analytical calculations and experimental iterations, based on the set needs and specifications, and on the manufacturing limitations and the selected material mechanical properties. Accordingly modify the selected assembling process, iterate and optimize the detailed parameters and select and optimize various surface treatments. If major complexities occurred, or if the applicable manufactured dimensions don’t fit into the set needs and specifications, then select another assembling process and another manufacturing process accordingly. If it still doesn’t work, get back to the embodiment design stage, select a different

material, iterate and repeat. If the manufacturing process resulted in material, surface, mechanical, optical and geometrical properties that fit into the needs and specifications then select an assembling process and modify the assembling parameters to optimize the assembling process. If it still didn't work select a different assembling process test and iterate, and if it still didn't work get back to the embodiment design, select another material, iterate and repeat. If the assembling process and treatments are optimized and fits well the needs and specifications, then test the assembly mechanistically through testing the microscopic properties using the SEM, then test the mechanical properties through compression and tensile tests, and the optical properties through the microscope and most importantly test the assembly functionally by performing leakage testing. If one of the tested results doesn't fit the needs and specifications, then perform further material and surface treatments, else select another assembling process and iterate, and if it still didn't work, select another manufacturing process, iterate and repeat, and if it still didn't work, go back to the embodiment design select another material, iterate and repeat the whole process until no leakage occurs. If no leak happens and the LOC assembly fits the needs and specifications mechanically optically and functionally, then perform biological functional testing, to test for the cell viability, adhesion and the experimental process. If cells are not viable due to toxicities or cell's incapability to adhere to the surface, then select another assembling process by removing the main source of toxicity or surface modifier, or by treating or coating the surface. If all applicable assembling processes didn't work, then get back to the embodiment design, select another material, modify, optimize the design dimensions accordingly, find the new design limits, iterate and repeat until the system fits all the needs and specifications, and the cells are viable and adhering to the surface. If the cells adhered to the surface, then adjust the biological experimental setup and modify and optimize its parameters, if the design have

biological experimental complexities, then modify the design by tackling the connections and the accessories of the design and not the design itself. If this still didn't work, then select another assembling process and iterate until those minor biological experimental problems are resolved. if it isn't resolved and core changes in the design are needed, then go back to the embodiment design, either for the same material optimize the geometrical parameters to resolve those problems, and if this isn't applicable, then choose another material and then iterate, and repeat the whole process until those problems are resolved.

The fifth and final stage of the design process is functionally developing and unleashing the potential applications of our design. At this stage, further biological experiments are to be done on the design, to develop experimental procedures for each of the applications. Initial applications would be optimizing the LOC for cell attachment on the inner wall of the duct and the formation of the full ductal tissue with cell polarity tested and proved to be expressed bio-mimetically. Afterwards, would be optimizing the experimental procedure for co-culture applications of the cancer cells inside the duct, in contact with the normal epithelial counterparts forming the ductal structure, and testing for the migration of those cancer cells through the duct to the other side. Then may come the co-culturing of the myoepithelial cells within the basement membrane surrounding the basal side of the epithelial cells forming the duct, and fibroblasts on the outside of the duct creating the stroma. And most importantly, the LOC would be tested on different drugs with known effect (both positive and negative controls) in addition to a new drug or component and modifying the experimental procedure for that. Finally, the system would be assessed experimentally and compared with a natural tissue biopsy, to shows the resemblance and the need for further modifications in the design or conceptually by enhancing the needs and

specifications. Afterwards, the LOC will be ready to be used by anybody, for various applications.

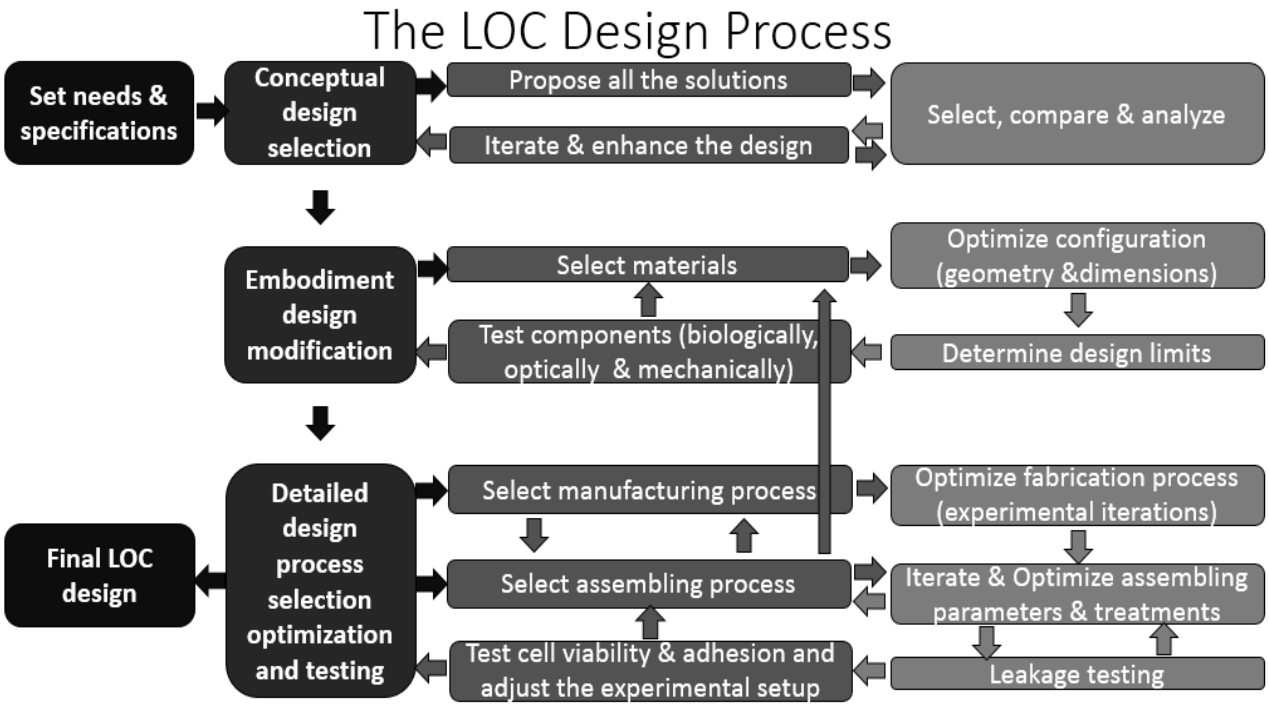


Figure 5 The LOC design process method of iteration

CHAPTER III.

LOC MODEL SPECIFICATIONS AND CONCEPTUAL DESIGN SELECTION

According to the literature on the biology of ductal tissues, cancer growth and metastasis from ductal systems, and current drug testing platforms and lab-on-chip designs for ductal tissues, there is a need for a better biomimetic platform for advanced in-vitro drug testing and research on ductal tissues [23].

The two most fundamental needs for the creation of a biomimetic model, are the cell polarity preservation through structural scaffolding & mechanotransduction and the co-culture cell signal exchange through the culturing of the epithelial tissue while in contact to the basement membrane, thus, being able to communicate and be molecularly signaled by the surrounding stroma [24]. Current systems have one of those two main aspects shows a major functional advancement and resemblance to the native tissue when compared to the regular 2D culture platform or 3D gels, but still shows many major differences when compared to the natural tissue. One main reason for the importance of the polarity preservation when testing on epithelial ductal tissues, is that polarized cells when formed into a duct they reach senescence, and thus stop to grow, in contrary to the cancerous counterparts that doesn't have a demarcated polarity and keep dividing and never reach senescence, leading to the cancer growth uncontrollably [25]. Thus, cells cultured and hooked on each other forming a close duct resembles the normal epithelial tissue rather than cells cultured in 2D that functions as cells at the duct formation stage grown in an abnormal environment that looks more like a cancerous environment than a normal one [26]. Co-culturing with the surrounding stroma is very important, as fibrous tissue plays an important role in the ductal cancer progression through hormonal and IGF levels changes causing changes

in the stromal cells gene expression, leading to changes in the extracellular matrix biomarkers and thus disrupting the signaling cascades from and to the epithelial tissues [27].

Another very important aspect that is needed for many different applications is the capability to test the drug's spatial effect on co-cultured cells. This means that for an assembled tissue, it is important to test the effect of a modification in a tissue environment on that of the surrounding tissue, and this is very important in cancer applications, especially due to the drastic change in the ECM leading to an alteration of the signaling pathways in the tissue cells and the cells in the surrounding tissues, and this was shown to play an vital role in cancer progression and metastasis [28]. In addition, culturing tissues with various properties and modifications while being in contact to the same tissue in the same system helps compare the effects of each, and study the progression stages of a disease and the synergistic effects of drugs and molecular components on various ductal diseases and most explicitly cancer metastasis and progression. Ductal cancers are complicated due to randomness in cellular expressions, for example, cancers grow and proliferate rapidly and express immensely different ECM components mainly collagen I, leading to a stiffer matrix, at the same time, cancer cells express different MMPs compositions leading to the breakdown of the matrix, leading to a less stiff ECM, in order to pave the way for the cancer grow, migrate and metastasize to different areas of the body. Thus, creating a system where the epithelial tissue is cultured and surrounded with a stroma having different components and stiffness at various locations, may help specifically identify the cancer progression stages, and what specific components effect this progression, and thus eventually find a way on how to control, block or reverse the cancer metastasis in ductal tissues [29].

Another important aspect to take into consideration when designing a new LOC device is the handling feasibility, as a design wouldn't be of an interest if it is complex to use, with very

limited applications and a hard or sensitive sterilization and handling process. For this reason, the design needs to be user friendly, versatile and easily sterilized. Versatility increases it chances as a successful potential market-level product, as it'll vastly increase the potential market size and costumer diversity. Being user friendly will make it more successful for market applications, since the potential users will most probably not have the knowledge and expertise in the design and engineering modifications of the product.

A. **Proposed Needs and Specifications**

For the conceptual design development, the needs and specifications of three main aspects are needed to be set. The first set of specifications is based on the structural needs of the design to fulfill the design aims included above, ensuring the creation of a ductal tissue where first, the structural and the co-cultural aspects of the design is preserved, second the special effect of the co-cultured cells could be tested and the ability to be user-friendly, versatile and sterilizable. The second set of specifications is based on the material needs and the ability to of the material to be fabricated into the set structural specifications, and its ability to be sterilized. The third set of iterations is based on the design adaptability specifications, which depends on the other two set of specifications, and is based on the applicability of a fabrication process on the designed features, and the assembling ability of the design features together and with the connectors with standardized structures and dimensions found in the market.

1. ***Structural Needs***

a. <u>Cells self-assembly into a duct:</u>

One of the two main goals of the design is the ability to self-assemble the cells into a circular cross-sectional ductal structure. This is feasible through either 3D printing the ductal tissue into the designed structure, knowing that this is expensive, time consuming and have many limitations, or through passing the cells in a micro-flow into the ductal scaffold with surface properties feasible for cell attachment at specific flow rates and cellular concentrations.

b. Feasible cell communication across the duct:

The other main goal of the design is the ability of the epithelial cells in the inner lining of the duct to communicate by exchanging various chemical and biological molecular signals. Thus, have the ability to create a controllable environment for a multi-parametric signaling pathway for cells to function as they do in the in vivo environment. This can be done by either culturing the two tissues directly in contact to each other, or by creating channels for the fluids in contact with both tissues to be exchanged.

c. Accessibility to all areas at all times:

One of the major advantages of in-vitro cultures is the ability to access different areas and levels of the cellular modifications at any time of the experimental setup, and this is important to understand better the time effect of a drug on the development of a cancer. But the problem is that the in-vitro cultures doesn't resembles the natural tissue micro-environment, and thus cells aren't assembled to form areas similar to the in-vivo, for this reason, this type of experiment doesn't give realistic information about what happens in the in-vivo. On the other hand, 3D cell culturing in gels produces 3D acini, but the problem is that this acinus is spherical in shape, thus the area of the lumen cannot be accessed. Few or now system exist today where both cultured areas can be accessed from both sides.

d. Thickness and dimensions for microscopy and handling:

A major structural limitation to take into consideration, is the thickness of the LOC, since most of the other dimensions are structurally dependent on this specific parameter. The thickness of the LOC depends mainly on the visualization capability of the current microscopes and on the handling capability and the manufacturability of the material.

2. *Material Needs*

a. Biocompatibility, chemical and surface properties

The most important aspect to take into consideration while choosing a material for a biological application is biocompatibility. The material that is to be chosen should not be toxic to cells and is preferable to have surface properties feasible for cell adhesion and cell growth. The cell adhesion aspect is very important as that if the cells cannot adhere to the surface, it will lose its motility, and eventually it will die, thus surface properties are very important in biological applications and eventually it will be the core of our application, as minor manipulation, treatment and coating of the surfaces ma play an impactful role on cells viability and attachment.

b. Mechanical Properties and manufacturability

The material that will be used should be feasible for various manufacturing applications, in such a way that it should be strong enough to withstand being cut to small dimensions, and the modulus of elasticity should be high enough for features with small thicknesses to be rigid enough to withstand the assembly without undesirable deformations. For applications where the design is to be fabricated through molding processes, then the strength and stiffness may not be the core mechanical parameters, rather the viscosity, melting or setting temperatures and other

fluid and thermal properties may be of a major importance. In addition, the material is preferred to be flexible to various treatments and modifications, in order to decrease the limitations and increase the potential and possibilities for the iterative optimization process, increasing our chance to reach an optimal design with least complexities.

c. Visualization Properties

Since most of the quantitative and qualitative experimental biology techniques are based on the microscopy and on the visualization of the cellular and molecular aspects of the setup, microscopy and visualization are the most important factor to take into consideration while designing an LOC device. For this reason, the designed setup should be made of materials that are transparent, thus light is accessible to pass through to the areas of cell culture. In addition, it is essential for the material to be applicable for immunofluorescence visualization applications, since many of the biological experimentations is based on the staining of specific molecular biomarkers in the cellular setup with fluorophores to visualize the presence and measure the concentrations of those specific biomarkers in the cellular setup. This is a major data collection resource for applications in cell and molecular biology, and currently cannot be replaced by other techniques.

3. *Design Adaptability Needs and Specifications*

a. Manufacturing Adaptability and Limitations

The design features and dimensions to be chosen should be manufacturable using current fabrication techniques. In a way that if the material used was to be fabricated by cutting techniques (drilling, milling, turning, threading, abrasion, among others), then the design

dimensions and features should be applicable to the sizes and geometries that those techniques can cut into, and to the standard sizes of the tools found in the market and is to be used. If the molding technique is to be used, then the viscosity of the melt and roughness and surface tension of the mold, should be taken into consideration when designing features such as the small areas and thicknesses and angles.

b. Assembling Limitations

Most of the manufacturing processes are limited to either one dimension of cut or to a specific thickness or depth of cut, and molds are limited by the closed areas or internal bulge areas. Thus, designs based on the assembly of different manufactured parts with each other are recommended. For this reason, it is recommended that the design could be made of different parts and the assembling of those parts using current techniques is applicable.

c. Adhesives or fusing Adaptability and Limitations

Most of the LOC assemblies, where single parts are manufactured through cutting techniques may require an adhesive layer to connect the parts together. The problem is that the design should be flexible for the usage of an adhesive, in a way not to risk the possibility for some design areas not to be reachable by the adhesive, or the possibility for a leak to happen to a different undesired area. Fusion or welding may also be applicable for many applications where assemblies are manufactured through cutting techniques but is essential for molding applications or applications where flexible materials, hydrophobic materials or similar materials are used. For this reason, design geometries should be applicable for this type of applications, and thus a significant area size is required to fuse different components together without significantly deforming the fabricated features.

d. Connections Adaptability and Limitations

The features that should be included in the design should have dimensions and geometries which are adaptable to the tubing and connectors found in the market. It is recommended that the features to be included in our design be applicable for frequently available products in the market, not for custom made products.

e. Other Adaptability and Limitations

i. Sterilization Limitation

The features, dimensions, materials and surface properties are important factors for various sterilization techniques. Material limitations may be the chemical incompatibility with ethanol, UV radiation or high temperatures of pressures for autoclaving. Dimensional limitations may be, the inaccessibility for UV, ethanol of steam to reach some fabricated areas of the design. And surface properties may be incompatible with sterilization techniques, in a way that it may be altered after a sterilization process is done, or the optical properties may be altered.

ii. Handling Limitation

The shape and the dimensions to be taken into consideration when designing the device should be feasible to ease the handling of this device when a biological experiment is being performed. For example, the size should be ergonomically compatible with the average human hand, and features such as sharp angles should be removed for safety reasons.

B. **Proposed Solution and Design**

The conceptual design process is an iterative process that is based on a loop that begin by proposing all the possible solutions to the problem, based on current designs in the microfluidics

field, and further useful designs in other related fields. In order to later select a conceptual design that is to be analyzed and compared with all the other set designs in an iterative process, and then enhanced and modified to fit all the set needs and specifications. If the enhanced chosen design fits all the needs and specifications, then the LOC would be ready to progress to the embodiment design stage, else the iteration should continue, where another design is to be designed, selected, analyzed, enhanced and then compared with all the other solutions until the optimal design is found.

1. *Proposed Solutions, Analysis and Design Selection*

The first step for the conceptual design creation and selection process after getting insight to all the current solutions being used in the field and in other related fields, is proposing basic initial designs for the problems that best fit into the set needs and specifications. Many designs are proposed and compared, and only three of them, described below, fits perfectly the set needs and specifications.

a. Design 1

The first design is based on a 1 step molding of PDMS or any thermoset or thermoplastic material into a structure having a circular cross-sectional channel that is open through few other micro-channels to two different areas that is accessible from another place. In this design, the epithelial cells could be pumped into the duct, and assembled into its walls, and the stroma could be pipetted and cultured into the other two open areas, and the micro-channels in between may play the role of biologically and molecularly connecting all the areas together. This design could be created using a press mold made of three main dynamic components, the shaft creating the area of the duct, the cubes or cylinders creating the areas of the stroma areas, and the needles issued from the cubes or cylinders areas to key fit into the shaft, creating the micro-pores.

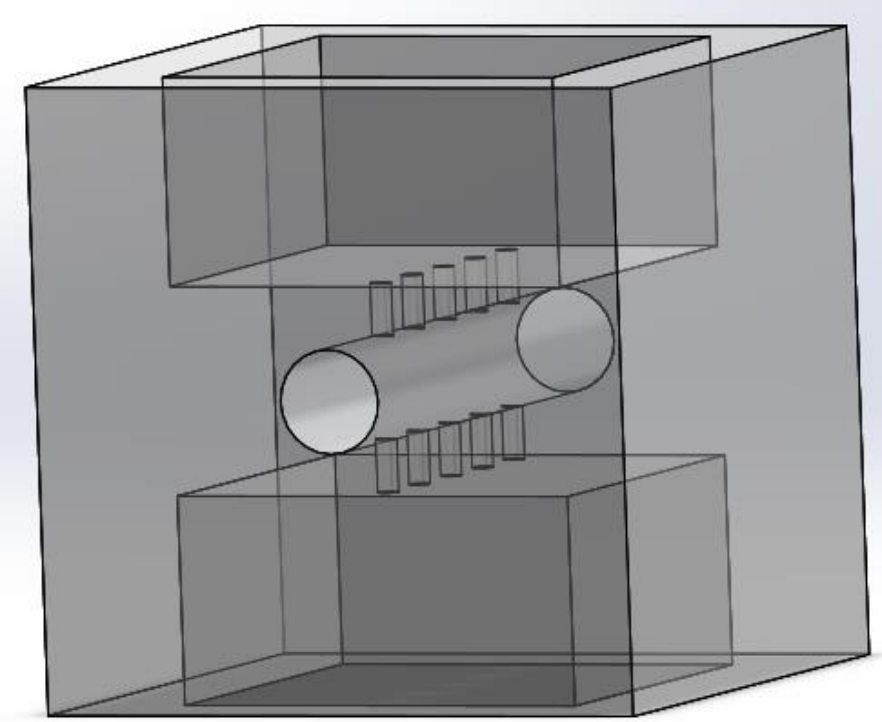


Figure 6 Conceptual design 1 CAD drawing

b. Design 2

The second design is based on a fabrication and assembling process of two flexible porous membranes, that each is to be deformed into a scaffold bounding its size and its deformation degree of freedom, in addition to being open from all around to an open area or well. In this design, the epithelial cells could be pumped into the formed duct and assembled into its walls, and the stroma could be pipetted and cultured into the other two open areas or wells, and the pores of the membrane may play the role of connecting the ductal area to the wells areas. This design could be created by fabricating each of the scaffolds on its own, either by cutting it using laser or milling machines, or by molding it, and the membranes could be purchased with the needed thickness, surface and porosity properties and cut into the specific dimensions and assembled into the scaffold, and then each of the scaffolds can be assembled to each other.

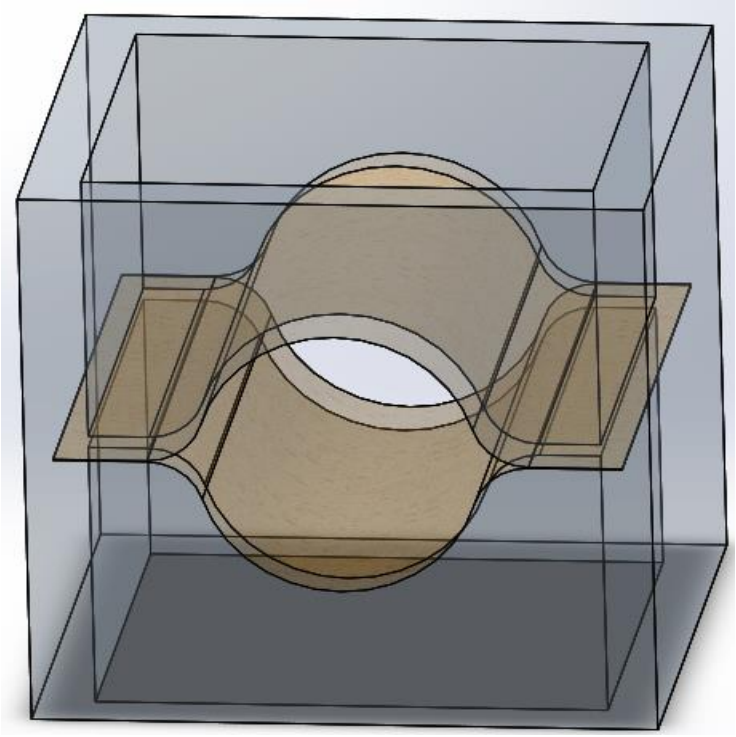


Figure 7 Conceptual design 2 CAD drawing

c. Design 3

The third design is a very simple design and is based on the fabrication or molding of a circular cross-sectional duct in plastic, glass, or PDMS. When used for biological experimentation. the stroma tissue would be assembled on the inner wall of the duct, and then biological adhesives may be used to adhere the basement membrane on the inner surface of the built stroma tissue, and then the epithelial cells may be passed and adhered on top of the assembled basement membrane, thus creating the full ductal system. The above adhesion between the layers could be done through using surface coating of the gels by fibronectin, PEGs, or other materials, or through the usage of nanoparticles adhesion process to connect the two gels together, noting that a French team had successfully created an adhesive made of silica nanoparticles to connect two gels together using the adsorption force between the molecular network of the gels and the silica nano-particles.

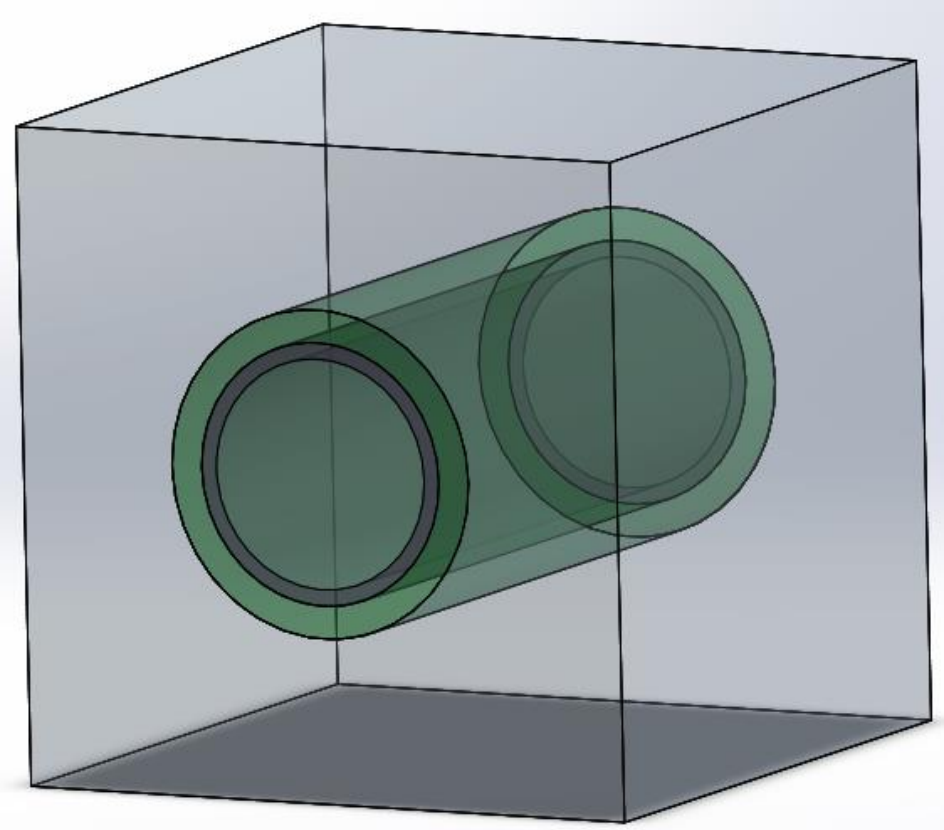


Figure 8 Conceptual design 3 CAD drawing

2. *Design Analysis and Selection*

The three proposed designs described above, are now to be analyzed and compared in order to select one of them accordingly. Factors such as the level of structural and cultural biomimicry, the feasibility to perform various migration assays, the applicability to broad applications, the accessibility to both areas of the duct at any time of a biological experiment, the biological experimental ease, the ease to manufacture and assemble the device, and the potential cost are taken into consideration while assessing each of the designs.

a. Design 1 Analysis

Design 1 is relatively cheap and have excellent structural resemblance to the real tissue, can be easily manufacturable into different structures, user can have good accessibility to both the inside and the outside areas of the ductal structure at any time in an experiment, In addition it can be used on a large number of applications. But it doesn't have a good co-cultural resemblance to the real tissue, since the inside and the outside areas of the duct are not in good contact with each other, as they are only in contact with each through a few and relatively deep micro channels, and thus migration assays from the stroma to the duct epithelial tissue or vice versa.

b. Design 2 Analysis

Design 2 have a good structural resemblance to the natural tissue since an almost complete circular cross-section can be formed by the two assembled deformed membranes. In addition, it has a good co-cultural resemblance to the real tissue, since the epithelial tissue that forms the duct is only separated from the stroma that surrounds it throughout, by a very thin highly porous membrane. Design 2 also have good accessibility from both the inside and the outside of the duct since both areas can be accessed at any given time of the experimental setup. Since the membrane is of a controllable pore size and density and of a very small thickness separating both areas of the duct, then countless number of migration assays is applicable, in addition to being user friendly in many experimental setups and can be used on a broad number of other applications. The problem is that it is relatively more expensive than the other two designs, since it requires a more complicated fabrication and assembling processes.

c. Design 3 Analysis

Design 3 is the cheapest, as it is the easiest to manufacture, in addition to having an excellent structural resemblance and an excellent co-cultural resemblance since both the epithelial and the stroma tissues are fully in contact with each other, and nothing is separating those two areas. But

on the other hand, it has a major limitation, which is that the stromal tissue cannot be accessed after assembling the tissue, and thus cannot be modified during the experimental setup. Another major limitation is that it is hard to assemble the tissues/ gels on top of each other, as either the flow will wear the pre-built layers off, and there is no standardized way to adhere gels on top of each other, and if nanoparticles or other adhesives were used, then the it may affect the experimental setup, as it may affect the experimental data.

d. Design Selection

After the three designs were analyzed, they are now compared with each other according to the set needs and specifications, and accordingly only one of the designs will be chosen to go with into the embodiment design stage. To begin, design 3 have a blurry potential, as it is not user friendly, it has many complexities and limitations for tissue assembly, especially for cell migration applications, and thus it will be excluded. Design 1 and design 2 are a bit similar in ideation, but since design 2 have a better co-cultural biomimicry and have more benefits and applications especially for cell migration applications, then we will choose to go by design 2.

Table 1 Comparative analysis on the conceptual designs

	Design 1	Design 2	Design 3
Structural biomimicry			
Co-cultural biomimicry			
Migration assays feasibility			
Applicability to broad applications			
Accessibility to both areas of duct			
Experimental ease			
Manufacturing ease			
Assembling ease			
Cost			

Design 2 was selected because it best fit the set needs and specifications, in which it has a very good resemblance to the real tissue, migration assays are applicable through it, it has good accessibility to both sides of the duct and can be easily used on broad applications.

The next step is adding the less important needs and specifications, thus preparing it for the embodiment design stage. At this level, our additional aim is to make the design applicable for high throughput applications and have access to different regions along each duct. Thus, we re-iterated the design according to the defined set of iterations (the main specifications and the additional ones). For this reason, we did 21 different iterations on the selected design (design 2), where we optimized various features in the design accordingly. We will describe only few of those iterations designs briefly below:

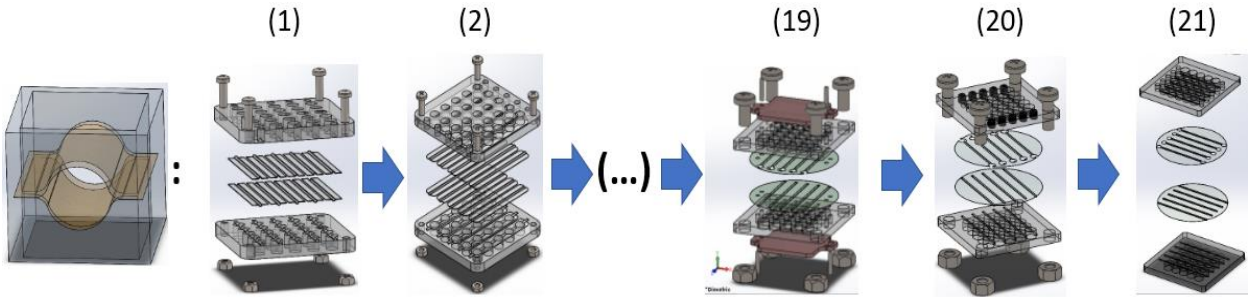


Figure 9 Selected conceptual design iterations (21 iterations)

Conceptual design iteration (1):

The first design that we choose to go by, is based on a chassis with five grooved hemi-cylinders, each with five holes drilled through it to act as wells. Then aligned on top of each of the hemi-cylinders is a very thin porous membrane, that is to be deformed using a formed press having hemi-cylindrical shaped extrudes, to deform the membrane and give it the shape of the hemi-cylinder in the chassis. This deformed membrane is held into position with the defined structure

bounded by 6 different areas formed by the hemi-cylinder groove crossing through the wells' holes. For each well area, the hemi-cylinder deformation is bounded and held at two areas, and the flat surrounding surface is bounded by the rest of the well's circular boundaries. The wells are relatively small in diameter and the proximity of the wells from each other is large enough to deform the membrane and bound it into the defined structure without a significant unfavored flexibility due to the malleability of the membrane resulting non-planned geometries. The edges of the chassis are filleted to remove the unfavored sharp angles, and the thickness of the chassis is chosen to be thick to make it strong enough as scaffolding for the membrane to hold it in the defined position. Bolts and nuts are chosen to force the surfaces against each other, and as a reference for the hemi-cylinders to align above each other creating a duct. A preliminary prototype was fabricated, the chassis fabrication process was simple, but the membrane cutting and assembly was very hard and time consuming. The main problem occurred was that when deforming the membrane open areas having the same thickness of the membrane would be open at the regions of the inlet and outlet, thus result in leakage.

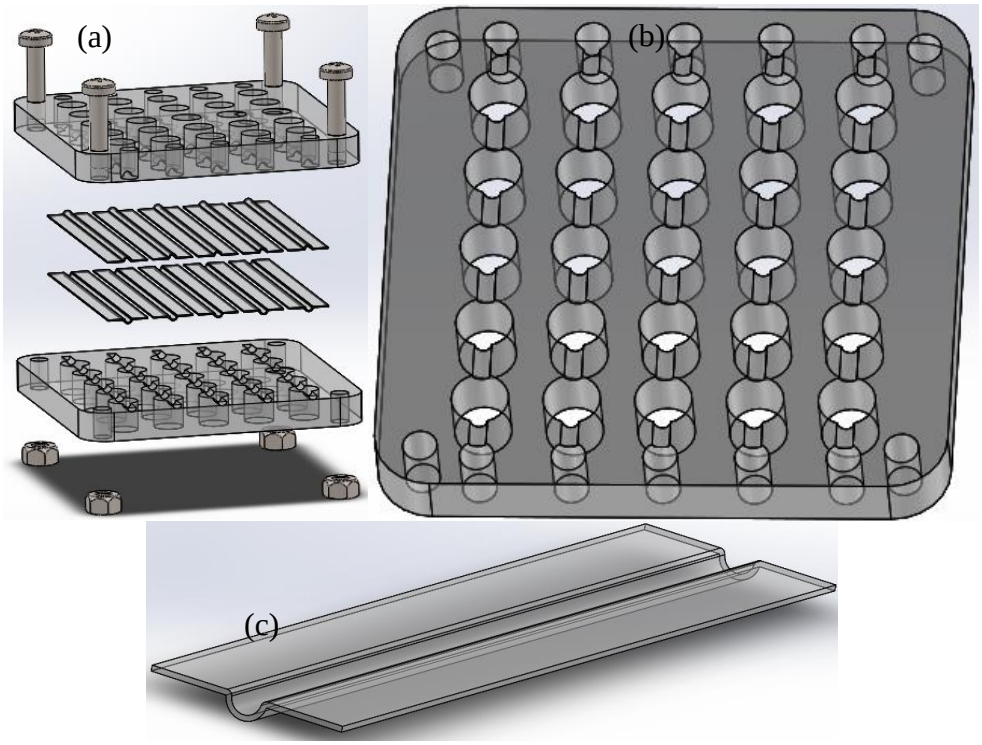


Figure 10 Conceptual design iteration 1 CAD drawing (a) assembly exploded view (b) Chassis (c) membrane

Conceptual Design (2):

The second design that we choose to go by, is similar to that of the first design but with one more feature. This feature is based on grooving the areas on the chassis surface under which each of the membranes would be located. This formed groove is as deep as the membrane thickness, have the length of the duct and the cut membrane, and as wide as that of the cut membrane when deformed. A preliminary prototype was fabricated, the chassis fabrication process was extremely complicated as the membrane is very thin (~10um), thus the groove should be of a very shallow depth (~10um). The CNC milling machine used to fabricate this preliminary chassis prototype have an x-y-z axis step of 3um which is significantly large relative to the groove aimed to be cut, thus resulting in significant errors when manufacturing, in addition to many complexities when zeroing and taking a reference point for each of the axis of cut, and when positioning the chassis

material to be cut. The membrane cutting and assembly was still very hard and time consuming. But the problem of leakage due to dimensional variances that used to occur, was solved and the membranes fit well in the grooves creating a more precise assembly. But the problem, is that the process is very time and money consuming, is susceptible to errors, and repeatability and reproducibility of the design wasn't an easy task since many parameters that needs precision plays a role, thus a different solution is needed. Another problem occurring that was also occurring in the first iteration design, but wasn't clearly shown due to the many other complexities, was that the ducts at the areas of the wells doesn't have a circular cross-section when fluid is passed in between, it rather have an elliptical or eye-shaped cross-sections, due to the flexibility of the membranes and the fact that it is bounded only by two areas surrounding each well, and the rest of the bounds are at the boundaries of the well itself.

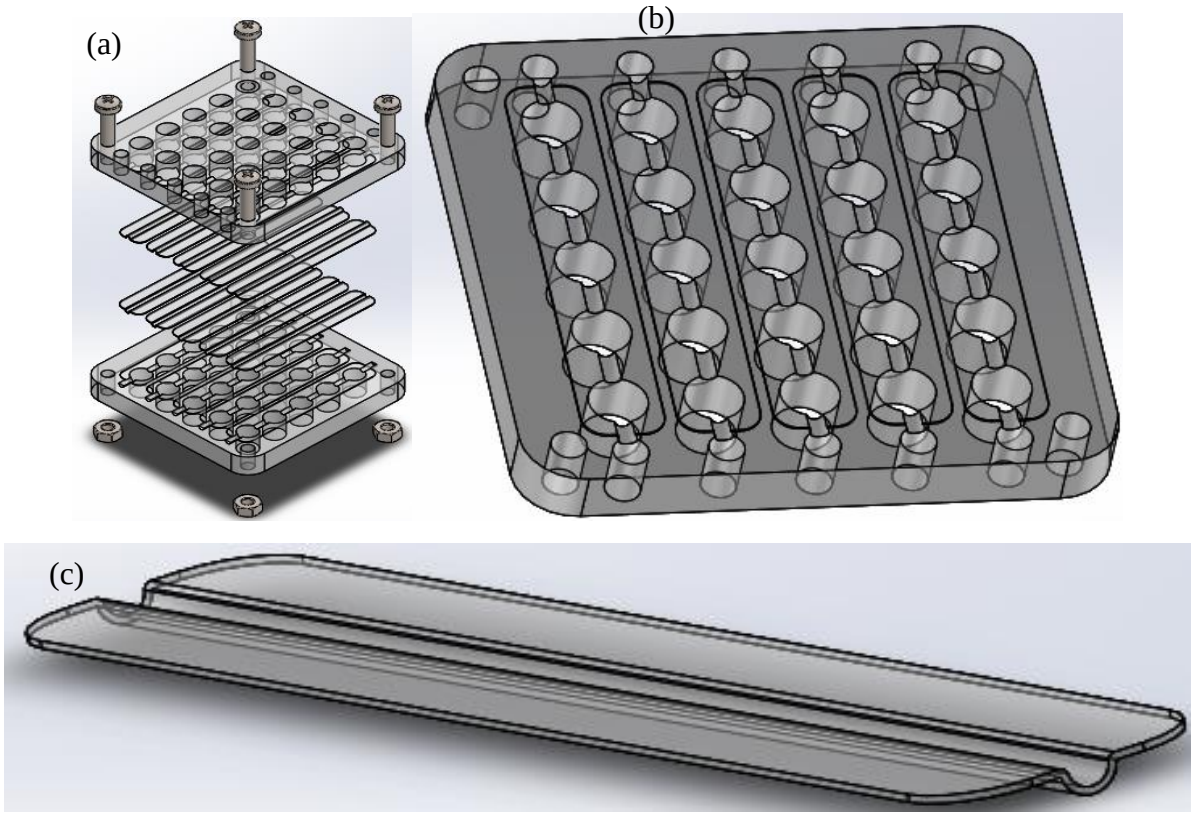


Figure 11 Conceptual design iteration 2 CAD drawing (a) assembly exploded view (b) Chassis (c) membrane

Conceptual design iteration (3) to (18):

The third design iteration that we choose to go by, is similar to that of the second design but with one additional feature. This feature is based on increasing the depth of cut of the hemicylindrical groove only at areas where the wells will be formed, and then rotating the chassis to the other side, and drilling the wells up to a proximity where its open to the deepened cut of the hemi-cylinder from the other side. The preliminary prototype of the chassis was then fabricated, and after many trials it had resulted in an astonishing observation, which is that the full duct was formed with no structural deformations of the membrane upon usage, an a circular cross-sectional duct was formed instead of an elliptical or eye-shaped one, since the duct diameter is bounded along its whole length by a chassis, and radially by the areas of proximity between the wells. In addition to the many complexities of the second design iteration, many additional complexities popped out. The first complexity was the fact that one more step was added while deepening the hemi-cylindrical groove, this was complex since a minor error occurring while positioning (such as non-coplanarity), or while zeroing and taking the reference x-y-z axis may happen, resulting in fault features. The second complexity was the fact that flipping the chassis material to the other side was required and thus re-positioning and zeroing was required to be done again resulting in many additional causes of errors.

The fourth design iteration that we choose to go by was similar to that of the third design, but with one modified feature for simplification. This feature is based on grooving the chassis the size of one large membrane covering all the ducts rather than grooving five areas for five membranes each used on one duct. The reason why this wasn’t used before is because of the probability that the membrane would break if deformed into different areas at the same time, due to the resulting tensile stress. Then a large membrane larger than the whole ducts will be

deformed in one single step into the whole five hemi-cylinders. After many trials, a preliminary prototype was created, and the large membrane fitted in the groove, and one step was required to deform it into the five different hemi-cylinders without breaking the membrane.

The fifth design iteration that we choose to go by was similar to that of the fourth iteration design, but also with one modified feature for simplification. This feature is based on the idea of removing the step where we used to groove the chassis at the area where the membrane would fit in, and on the other hand either use a membrane large enough to cover the whole features of the chassis for no leakage to happen, or reduce the size of the chassis so that all the features would be covered by the membrane. Using this process, the two chassis parts will not get in contact with each other, rather the only areas of contact between that upper subassembly (chassis and membrane) and the lower subassembly (the other chassis and membrane) is the contact between the membranes. This iteration had reduced one step of complexity which is grooving a very shallow depth of cut on the chassis surface to fit the membrane in, since the membrane is very thin, and this process is very hard, expensive and is susceptible to many errors when using current fabrication techniques.

The sixth design iteration that we choose to go by was similar to that of the fifth iteration design, but also with one modified feature for simplification. This feature is based on the idea of removing the step where we use to deepen the groove of cut of the duct at the areas of the wells when creating the hemi-cylinder, and on the other hand increase the depth of cut of the wells drilled from the other side to reach an area where the duct and the wells are open to each other at the level of the wells. This iteration had reduced one very complex step that used to result in many various errors.

The seventh design iteration that we choose to go by was similar to that of the sixth iteration design, but also with one additional feature. This feature is based on the idea of covering the wells from above and below with cover parts that are connected to the chassis with screws. This step is important to ensure that the wells are covered when a gel or media is added to it, so it is held in position and don't fall.

The eighth design iteration to the eighteenth design iteration, are technical feature iterations, and are based on choosing a specific technical feature to go by instead of the other based on the better mimicry, manufacturability limitations, the market limitations and the precision improvement. For example, the wells are got closer to each other in one of the iterations, to reduce the areas where cells that are to be grown on the inner sides of the duct are not surrounded with stroma from the other side of the duct, but rather by plastic. In another iteration, the bolts and nuts used to connect the two subassemblies together, are got closer to each other and to the other features to reduce their effect on the bending of the chassis. In another iteration, the diameter of the bolt chosen, and the hole drilled for it, is reduced in order to reduce the backlash of the bolt in the holes and thus create a better and more precise alignment of the two hemi-ducts above each other to create a full duct. In another iteration, the screws that are used to connect the wells covers with the chassis was replaced with pins since they require less volume.

Conceptual design (19):

The nineteenth design iteration that we choose to go by was a modified version of the other iterations, with two additional features. The first feature goes by changing the shape of the wells' cover in order to be able to manipulate and remove each cover at a time without being dependent

on the other cover. In order to do so, the cover is cut into a Z-shaped plate with two areas for the pins to connect the covers with the chassis. For the second feature, a round bolt was used instead of a square bolt, because it has a smaller outside thickness.

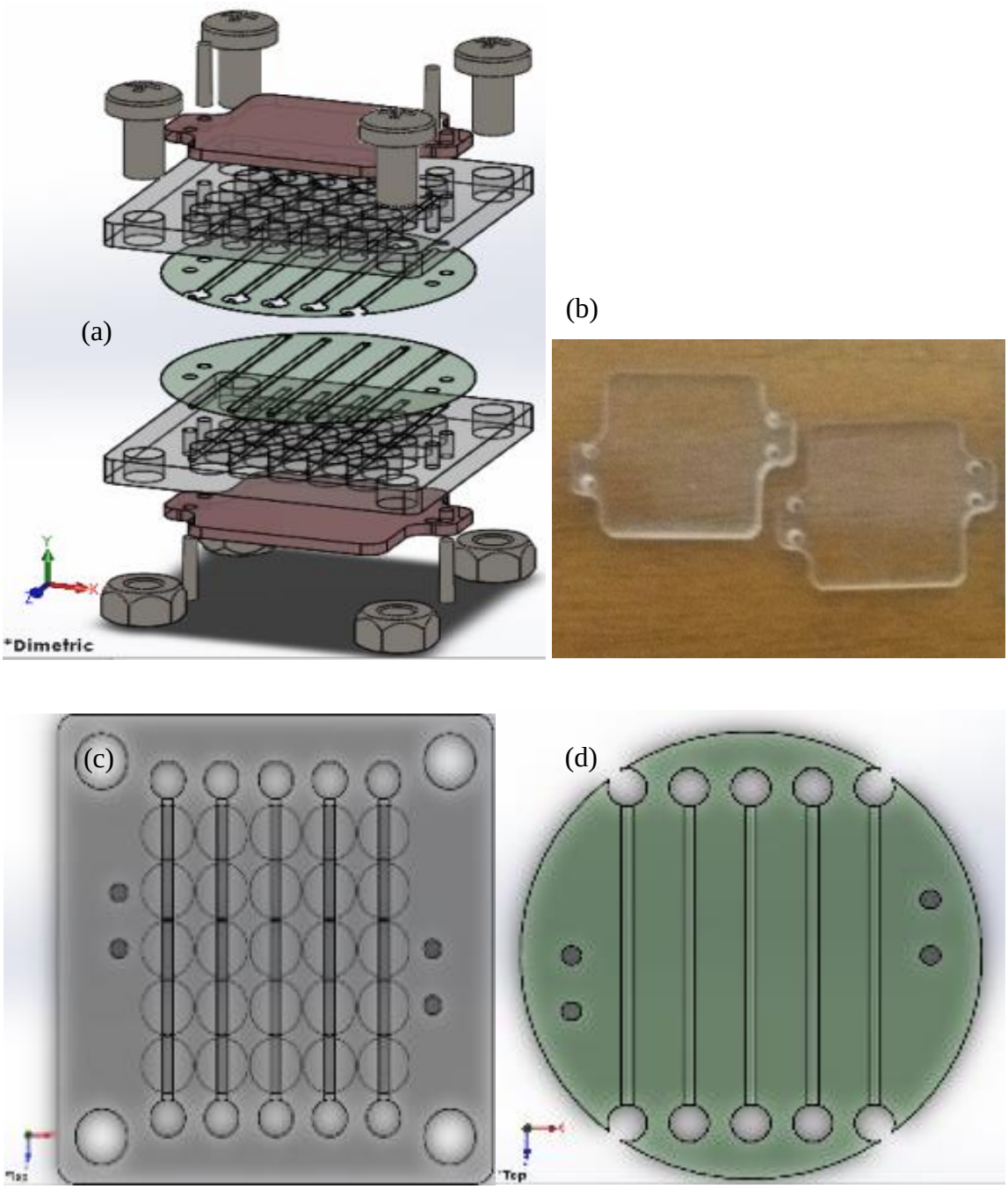


Figure 12 Conceptual design iteration 19 CAD drawing (a) assembly exploded view (b) PMMA well covers (c) Chassis (d) deformed membrane

Conceptual design iteration (20):

The twentieth design iteration that we choose to go by, was similar to the nineteenth design iteration, but with two additional features. The first goes by threading the inlet and outlet holes for threaded tubing connectors to fit well in it, this was after having a problem to tightly connect the tubes inside the holes. The second feature is based on using a coverslip and adhering it on top of the chassis, in order to cover the wells instead of using pins or screws to prevent cross-contamination between the wells that may result from leakage of liquid from one duct to another.

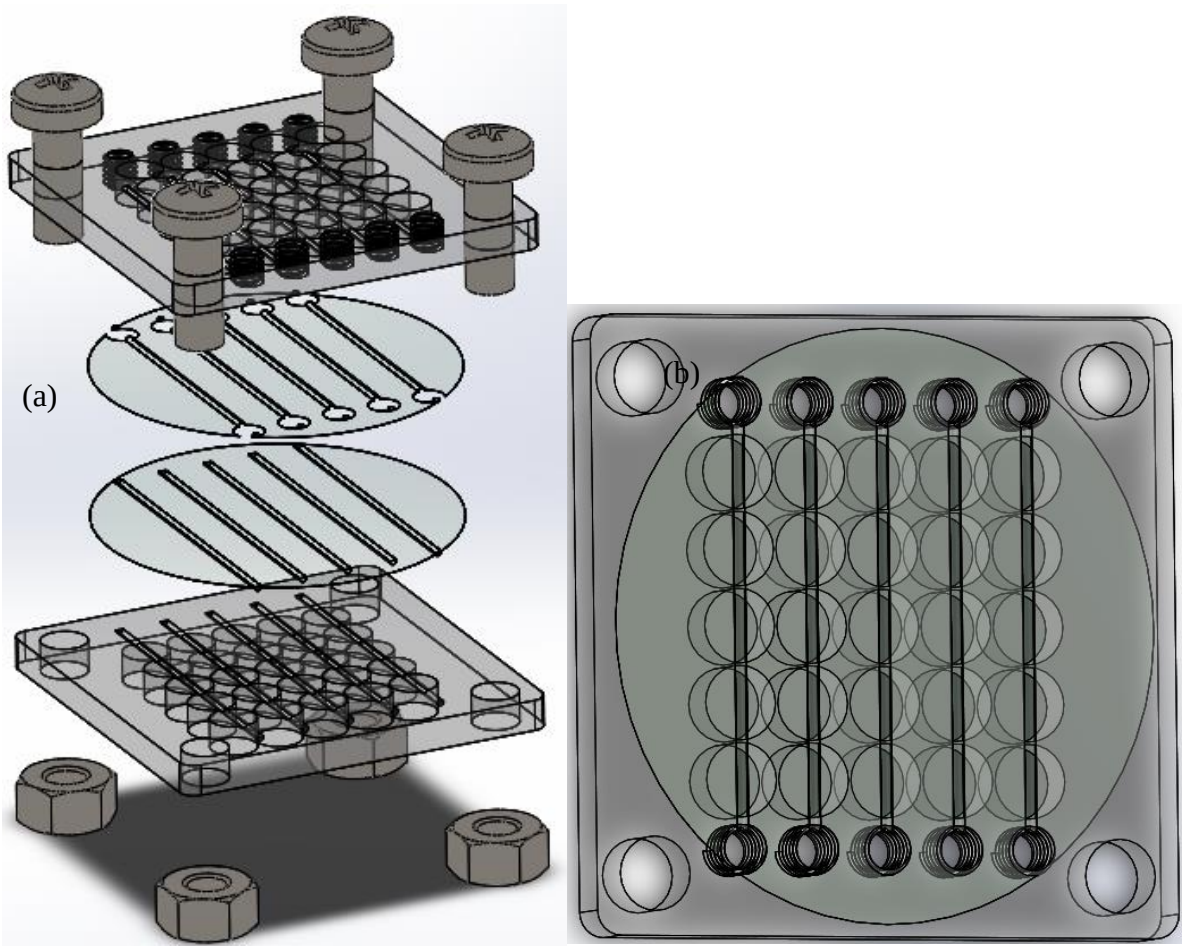


Figure 13 Conceptual design iteration 19 CAD drawing (a) assembly exploded view (b) Assembled view with threaded chassis inlet and outlet holes

Conceptual design iteration (21):

The twenty-first design iteration was the last conceptual design iteration that we did, and it only differs from the twentieth iteration design by two features. The first feature was by not choosing to thread the inlet and outlets holes, but instead choose to the flexible tygon tubes instead of the tough Teflon tubes, the reason was because the threads wears out for the tough plastic materials, only few can have strong threads, and elastic tubes cannot be threaded. The second feature was not to drill holes in the chassis to use bolts and nuts to connect the two chassis parts together and calibrate the ducts locations, because of the low precision of this method. Instead we used a plastic wire having a diameter equal to the inner diameter of the duct that is to be formed by the membranes. This wire will force the two hemi-cylinders to assemble above each other precisely. At this level, the LOC was ready for the embodiment design stage, to choose the optimal materials and dimensions to be used.

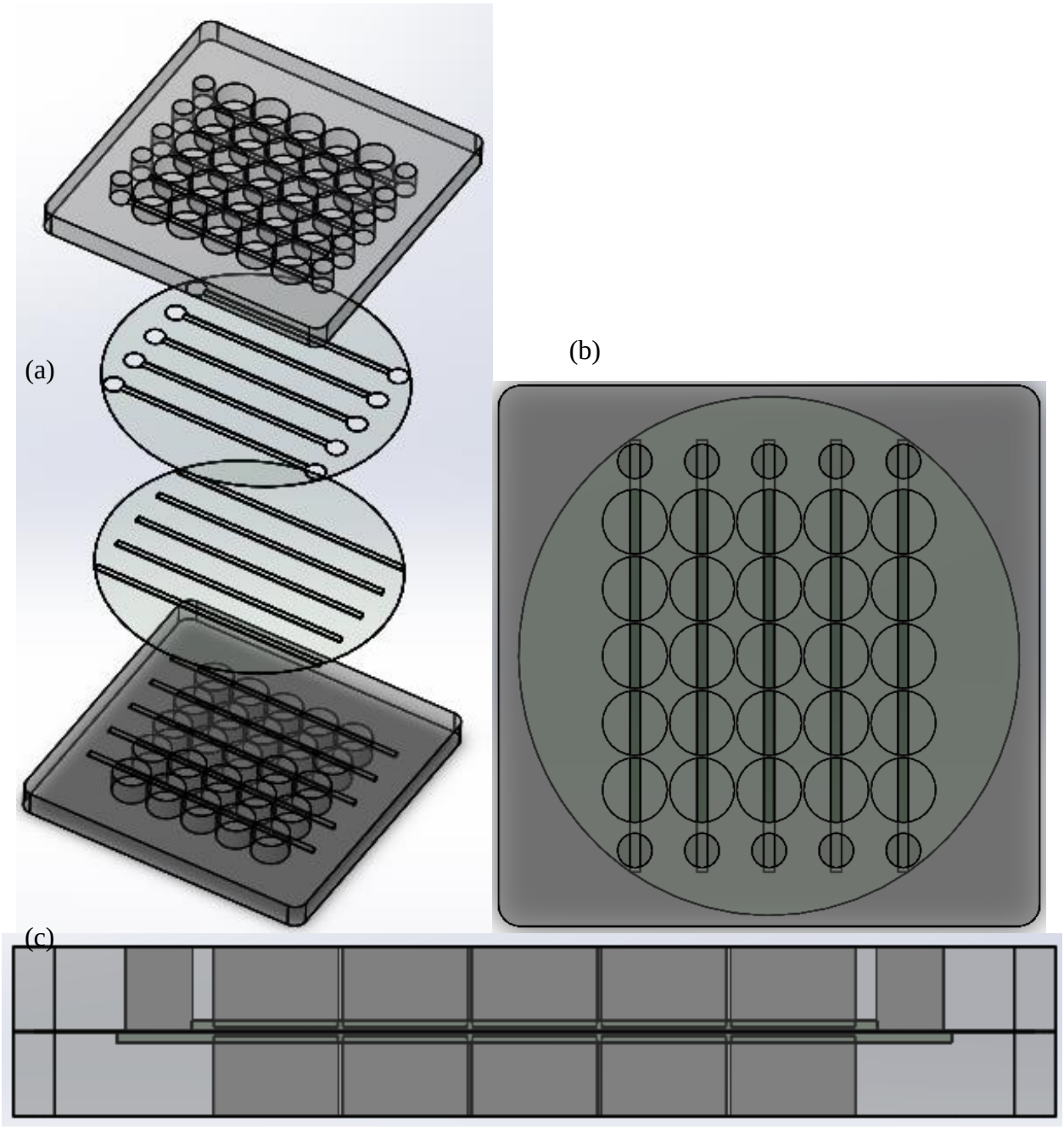


Figure 14 Conceptual design iteration 19 CAD drawing (a) assembly exploded view (b) Assembly top view showing ducts, channets, inlet & outlet holes with no threads and no bolts and nuts (c) Assembly side view showing the inlet & outlets holes, the wells from both sides and the duct in between.

CHAPTER IV.

EMBODIMENT DESIGN PARAMETERS SELECTION AND OPTIMIZATION SPECIFICATIONS, CRITERIA AND RESULTS

A. **Preface on our finalized conceptual design**

After setting the final conceptual design iteration as described in Chapter III, it is important to compare it with the real tissue, in order to see to what extent, it is biomimetic, in terms of specific features, and to get an idea on what can be done to enhance the design. In addition, comparing the defined design with the real tissue is important to know the current limitations of the design, and to set further needs and specifications to go by for the embodiment design, without pivoting from the main objective of the design.

Looking at the cross-section of each of the ducts created using our LOC, we can clearly see that ideally, the epithelial cells can be assembled on the inner walls of the duct-forming membrane, similar to the real tissue, after coating it with basement membrane material. In addition, the stroma can be seeded with all its components on the other side of the duct, thus completely surrounding the duct-forming membrane and thus is only in contact with the basement membrane and the epithelial cells through the high dense pores of the membrane. The pores are an important aspect of our design, as it is important to define the pore size, density and depth to set a limitation for the normal cells to pass through it but give a chance for invasive cells to deform and pass through the pores to the other side of the duct, thus resembling what happens in the invivo.

Looking on the top view, and the top cross-section of the duct, we can see that similar to what we see when looking at the duct cross-section, in addition to the fact that cancer cells grow on the inner walls of the epithelial tissue inside the duct and then it migrates to the other side,

depending on the growth of this tissue, and on the signaling complex that comes from the cells, the basement membrane and the stroma, to facilitate the migration of the cancer to the other side of the duct to grow there and then metastasize to other areas of the body and build colonies. Knowing the fact that all this complex buildup is important in defining the pathway for cancer migration, then it is important to design a system that can test the effect of all the possible combinations and compare its effect, and test the synergistic effect of different complexities, which mimic what happens in the in vivo because of the randomness in the cellular expressions of the cancer cells. For this reason, the design was created in a way to allow build different tissue complexes along the same duct, thus being able to compare the effect of different complexes and to study the synergistic effect of various molecular properties of the ductal structure. The design goes by creating different separate areas to assemble the stroma surrounding the duct, without caving access to each other except through the duct. In this way, many new parameters such as the likability of a cancer to migrate to a specific tissue and not the other can be tested. In addition, the synergistic effect on epithelial cells getting different signaling parameters at the same time can be studied when assessing cell proliferation, differentiation and migration. Not to forget the applications of intravasation from the stroma in a specific well to the duct, and then the extravasation from the duct to another well when feasible.

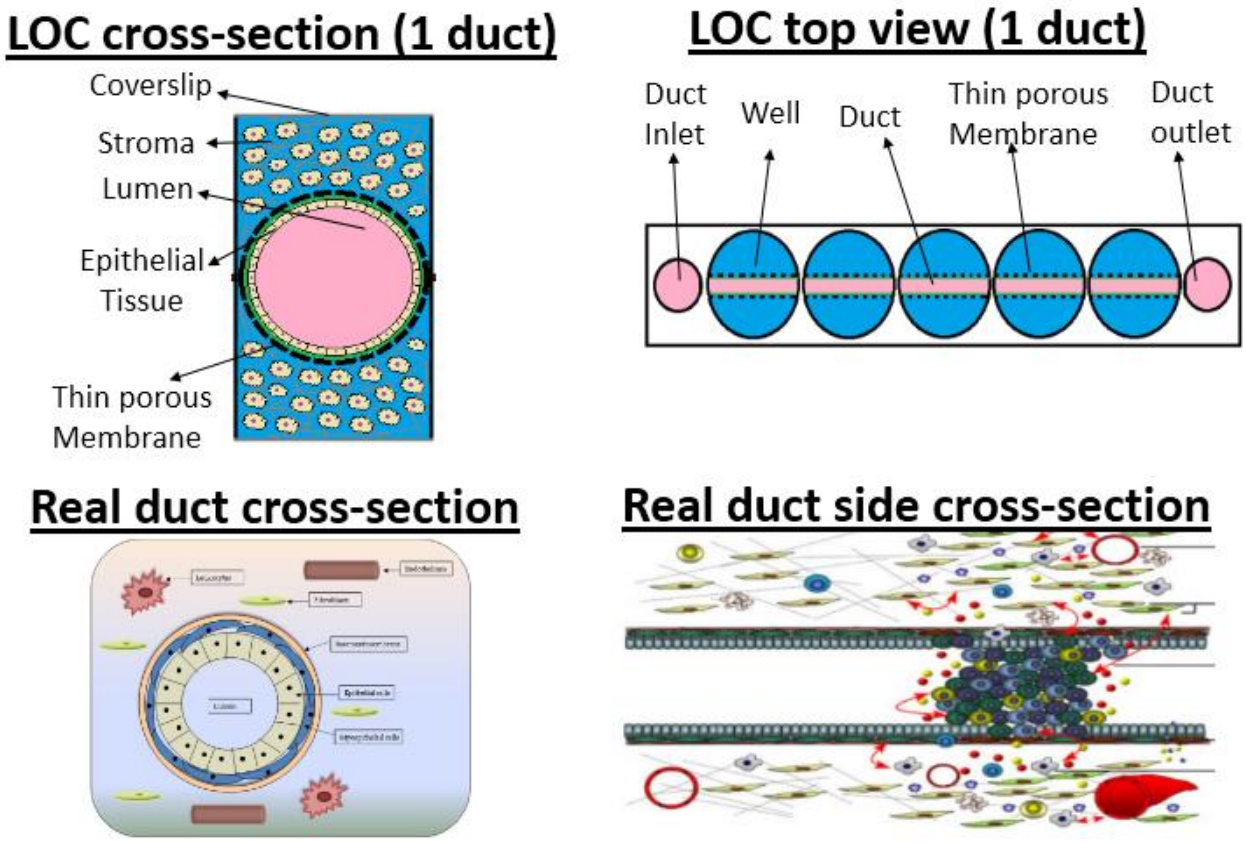


Figure 15 Comparison schematic of our design to the in-vivo (a) shows the LOC cross-section (b) shows the LOC top view of one duct (c) shows the real duct cross-secton (c) shows (d) shows the real duct side cross-section and cancer cells at their place of emergence

B. Design Parameters Optimization Criteria

1. *Material Selection*

Material selection in a design problem is the vital backbone in which all the other design parameters depends on. The material selection for each subpart of the design depends on the design needs and specifications (described in CHAPTER II), and the effect of the material of one subpart on other subparts and on the final product. Our design consists of four subparts, two chassis parts and two membranes, in addition to an adhesion connection in between the different

layers, which depends on the method and type of adhesion to be used (described in "Assembling Process Treatments and Optimizations" section below). The methods of selection of the materials of those subparts are described in thoroughly below:

a. Membrane Material needs and specifications

According to the final conceptual design iteration, and to the general specifications that we have for the LOC (set in Chapter II), we have a well-defined process to establish a more detailed set of specifications for the selection of the materials for the embodiment design of our LOC. According to the last design iteration, we need a transparent membrane that is flexible enough to be deformed into a circular cross-sectional hemi-duct, and with a specific porosity to enable the exchange of fluids from both sides of the duct, and for the cell migration across the duct in some cases. But additional specifications and sets are needed to be defined, most importantly are the material properties itself, that enable the defined aim to happen. Such properties are the mechanical properties of the membrane that is affected by many different restrictions, and the chemical properties that plays a role in the assembly of the LOC and the surface properties for cell attachment, and the optical properties that plays a role in the quality of the images that can be taken while using our device, in addition to other properties such as the biocompatibility, availability in the market, and some specific features such as the pore size and density. The following properties are discussed thoroughly below:

i. Mechanical properties

As stated before, the main aspect of our design is the feasibility to deform a very thin membrane into a ductal structure without breaking or plastically distorting the membrane. Thus, the membrane should have a high elastic strength preferably larger than 70 MPa (because of the low elastic modulus needed), and a tensile elastic modulus preferably smaller than 1 GPa, in order to

be very flexible for structural manipulations. The elastic modulus can be up to 3 GPa, if the material can be manipulated through thermal treatments to decrease the modulus while treating and deforming, without affecting the pore structure and size. For large elastic modulus > 3GPa, on a membrane having a thickness around 10 um, it would be hard to deform the membrane and keep it in shape while heat treating at relatively low glass transition temperatures, this may require higher temperatures, which may affect the thickness and pore size. Additional properties that are necessary are the thermal properties of the membrane, since we need a material that is thermally stable for all the temperature range of use (-20C till 150C). In addition, having a glass transition temperature low enough (preferably below the room temperature <25C) and a melting temperature high enough (preferably >160 C) but most importantly far from each other enough makes it unable to easily manipulate its other mechanical properties and structure.

ii. Chemical properties

Another main aspect of the material is the chemical properties of the material, allowing it to be resistant to the experimental conditions that may be done on the LOC, while having surface properties compatible with the conditions of the cells that are to be assembled on top of it. Most importantly, the material should be oxygen resistant, since it'll be used in environments rich in oxygen, and being in contact with cells, it is important not to degrade and release toxic ions that may affect the experimental setup if not being toxic to cells. Another chemical aspect to take into consideration is the solvent resistance of the material, since many solvents will be used in a biological experimental setup, most importantly the ethanol resistance, since in many cases ethanol will be used to sterilize the device. In addition to the surface properties of the material, this is an essential aspect, since it is the area that will be in contact with the cells and its matrix. One very important surface property is the surface charge that defines the hydrophobicity and the

hydrophilicity of the surface, a mainly hydrophilic material is required for the cells to be able to attach on top, thus a charged surface with contact angle less than 90 degrees is required.

iii. Optical properties

The optical properties of the materials are also an essential aspect while choosing a material for the design, since even if we found an optimal material for the cell growth and assembly, if it wasn't good enough to assess visually, it is useless because if so, most of the data cannot be extracted from it. Thus, the material shouldn't be just transparent, it should have minimum blurriness, and most importantly, it should have minimum auto fluorescence, since most of the experimental data will be assessed though a fluorescent microscope, after staining some specific biological molecular markers with fluorophores. Auto-fluorescence may interact with the experimental data and block interferes in most of the fluorescent signals of the experiment.

iv. Other properties

Other properties such as the biocompatibility, bioactivity and bio-functionality are very important parameters to take into consideration when choosing a material for the membrane, since the cells are required to live in contact to it and adhere to it. Additionally, the membrane should be sterilizable, preferably in more than one sterilization method to increase the chances to fit it with that of the material of the chassis. Moreover, it is essential for the membrane to have a pore size and density of 8 um and 1 cell / 100 um2, since it is the optimal pore properties for epithelial cell culture and invasion assays. And of course, the selected material should be available in the market with all the set properties.

b. Chassis Material Needs and Specifications

The chassis is the backbone of our design, it is the part that holds everything together and sets boundaries and structural limitations on which other parts (Membranes, tubing, fittings and

connectors) depends on to fulfill the structural needs of the design. In addition, the chassis is the part that contains the wells (the areas to be filled with materials that surround the duct) and the inlet & outlet openings, which are delicate parameters that sets many limitations to our design. Thus, the method of selection of the chassis material depends on the following parameters:

i. Mechanical parameters

- Young's modulus

The Young modulus is the material modulus of elasticity, it is the measure of the material ability to withstand a strain (change in length ratio) while under stress (tensile, compressive, shear or bending). The young modulus is the measure of the material's elasticity and tells about the way on how it can handle various loads. A rigid material (high Young Modulus E >1 GPa) is needed to hold all parts together with minimum deformation.

- Micro-fabrication technique and profiling feasibility

The fabrication process ease, cost efficiency, multilayers assembly capacity and profiling capacity (applicability on complex 3D structures) are the most important parameters needed to create a high-throughput fabrication of cheap microfluidic devices. The most common micro-fabrication techniques are photolithography, micro-milling, casting, photo-polymerization and thermo-molding.

- Thermal properties:

Thermostability is the material resistance to chemical and physical decomposition at relatively high temperatures. Thermostable plastics are usually thermosets since they cannot be reshaped, although they may be degraded. Thermostability is an advantageous aspect since it ensures stability of the material after various thermal sterilization (autoclaving) repetitions.

Thermo-fusing is a welding process used to fuse the surfaces of two thermoplastics together. Thermo-fusing is very important since it gives the material the structural flexibility and gives the user more control over the processes and more treatment and assembling options.

In addition, the operating temperature range for the material, and the glass transition temperature and the melting temperatures for thermoplastics is very important to consider.

- Structural limitation:

The smallest channel/feature dimension that can be formed by the selected material should be take into account (should be < 200um and preferably <50um). The limitation could be in the low strength of the material preventing the formation of small features, or the fabrication limitation, so that there is no fabrication process on the selected material that could do the needed small features.

ii. Chemical properties

- Oxidation resistance

The material should have a very good resistance to oxidation, since the environment that the LOC would be used in is highly oxidized. An oxidation in the material means that not only the material would corrode, but it will also affect the experimental parameters.

- Solvent compatibility

Chemical resistance is a vital aspect of the material, since the media that will be passed through the LOC is rich with glucose and many other components, and with varying acidity. In addition, the chemical sterilization methods that include cleaning with solvents such as ethanol are also to be taken into consideration.

- Oxygen permeability

Oxygen permeability is a significant parameter, but the need for it depends on the design, the thickness of the material and the areas of contact with the tissue. In addition, it depends on the mechanisms of the oxygen transfer to the tissue (through convective, conductive or diffusive mass transfer of the oxygen containing fluid).

- Surface adhesion

The surface properties of a material are the most important aspect of the design, since it defines the interaction of the material with all the materials in contact with it (the chassis, membrane and adhesive) and especially the cells and hydrogels and the tissue grown on the surface of the material.

The vital aspects of the surface properties are the hydrophobicity and hydrophilicity of the surface and the surface charge stability. The surface should be hydrophilic for good wetting and surface contact to occur; else it should be feasible for surface treatment to become so.

iii. Optical parameters

- Auto-fluorescence

Auto fluorescence is the natural emission of light from a material when they absorb light, and it is different from the light originating from the artificially added fluorophores. Many molecules and biological components have auto-fluorescing properties, such as the extracellular matrix (collagen and elastin), and amino-acid rich proteins (tryptophan and tyrosine). In fluorescence microscopy, auto fluorescence is problematic, since it interferes with the artificial light-emitting fluorescent stain signals bound to antibodies and used to visualize specific structures.

- Transparency

Material transparency is the property of light being passed through a material without being scattered, where the wavelength of the light is much smaller than the microscopic features inspected. In crystalline materials transparency depends on the long-range order of atoms, while that of glassy materials depends on composition and density fluctuations within the material. Good material translucency is needed for the chassis, but not necessarily full transparency, since it is not in contact to and neither blocking the area where the tissue is grown.

iv. Other properties

Other properties such as the biocompatibility, is very important, since cells will be in a near proximity to the surface. Bioactivity and bio-functionality are not as essential as in the membrane selection process, since cells aren't required to adhere to its surfaces (they will be suspended in gels) so it can be also bio-inert. Additionally, the chassis material should be sterilizable, preferably in more than one sterilization method to increase the chances to fit it with that of the material of the membrane that we have a limited number of choices when choosing it relative to the chassis materials options. And of course, the selected material should be available in the market with all the set properties and not just a niche or a custom-made product.

c. Analysis and selection

After setting the needs and specification for the materials to be used for each of the membrane and the chassis parts (as summed up in the table below), a list of potential materials with all its needed properties is formed and analyzed in order to select the optimal material for each of the components of our design.

Table 2 Materials needs and specifications for LOC

Chassis				Membrane			
Mechanical	**Chemical**	**Optical**	**other**	**Mechanical**	**Chemical**	**Optical**	**other**
E> 2.5 GPa Brittle	Oxygen resistant	transparent	Biocompatible	**E<1 Gpa Ductile**	Oxygen resistant	transparent	Biocompatible
Fabrication 3D profiling feasibility	Solvent resistant	Min auto-fluorescence	Available in market	**flexible (Features = 50um) Preferably tg<25C**	Solvent resistant	Min auto-fluorescence	Available in market
Operating temp: -20C till 150C Thermally stable	Surface charge Contact angle< 90			Operating temp: -20C till 150C Thermally stable	Surface charge Contact angle< 90		**Pore size = 8um Pore density = 1 cell / 100 um2**

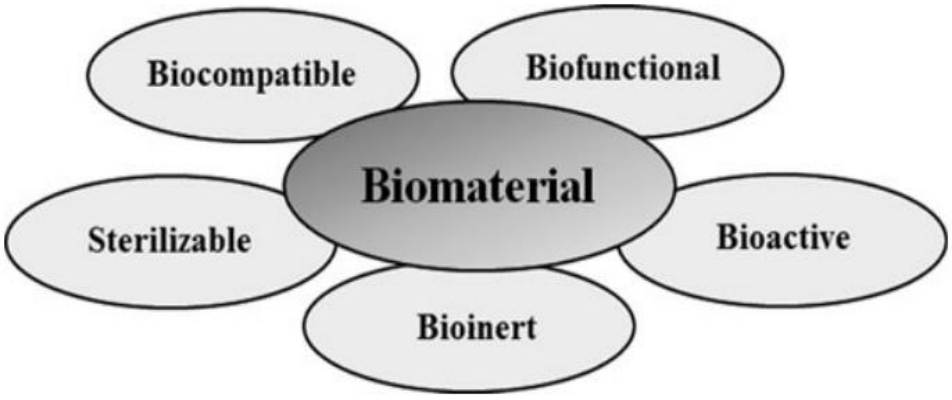


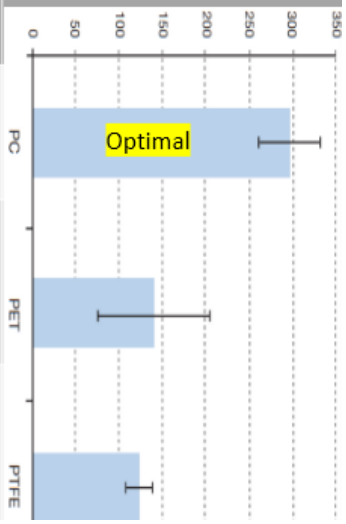
Figure 16 Biomaterials fundamental properties

A list of the common membrane available in the market and fit into the set needs and specifications (defined above), are compared according to the most vital mechanical optimal and chemical/biological (cell adhesion) parameters. For the mechanical properties, the modulus of elasticity, the ultimate tensile strength and the glass transition temperatures and the melting temperatures are assessed, where the strongest (higher ultimate tensile strength), most flexible (lowest modulus of elasticity), the most thermally flexible material for heat modification (lowest tg and highest tm) are assessed. For the optical properties, the fluorescent imaging quality was assessed for minimum auto fluorescent focusing problems and haze or blurriness. For the biological/chemical properties of the membrane, cell adhesion was assessed according to

experimental evaluations performed by the company (corning) that creates those membranes on HEK-293 cells. Three membranes (polycarbonate (PC), Polyethylene terephthalate (PET) and polytetrafluoroethylene (PTFE)) are selected for analysis as they fit the preset needs and specifications, and then compared accordingly. For the Modulus of elasticity, PTFE membrane was the optimal, as it has the lowest modulus E= 0.4 GPa compared with PC having E= 2.6 GPa and PET having an E= 2.7 GPa. For the ultimate tensile strength, PET membrane was selected, as it has the highest strength σu= 55MPa, compared to PC having a σu= 53MPa, and PTFE having σu= 15MPa. For the melting temperature and the glass transition temperature, PTFE was selected as it has the lowest glass transition temperature Tg= -97C and the highest melting temperature Tm= 320C and a thermal difference between Tg and Tm of Td= 417C, while PET had a Tg= 81C Tm= 264C and Td= 173C, and PC had a Tg= 147C Tm= 157C and Td= 10C. For the optical properties PET was chosen as it had the optimal fluorescent imaging quality, since it showed the maximal transparency, and minimal auto-fluorescence and focusing problems, while PC showed a good imaging property but with slight haze affecting on the imaging quality, and PTFE showed inferior imaging properties with problems with focusing and material autofluorescence. For cell adhesion, experiments performed on HEK-293 cells performed by corning, had shown that PC had the optimal cell adhesion properties, with the maximal binding and proliferation of cells, while PET showed average cell adhesion properties, and PTFE showed minimal cell adhesion. Comparing all the set together, we rejected PTFE as it has minimal cell adhesion and optical properties, compared to PC and PET, although it has optimal mechanical properties in terms of elasticity and temperature. And PC was also rejected for its average optical properties and non-flexible thermal properties, although it has optima cell adhesion properties.

Thus, we choose to go with PET as it has optimal imaging properties with good cell adhesion and mechanical properties.

Table 3 Membrane materials listing, analysis and selection

List of biocompatible transparent porous membranes found in the market			
Material	**Mechanical**	**Optical fluorescence imaging quality**	**Cell adhesion**
Polycarbonate (PC)	E= 2.6 Gpa σu= 53 Mpa Tg= 147C & Tm=157C	Good (Slight haze)	
Polyethylene tetraphalate (PET)	E= 2.7 Gpa σu= 55 Mpa Tg= 81C & Tm= 264C	Optimal	
Polytetrafluoro-ethylene (PTFE)	E = 0.4 Gpa σu= 15 Mpa Tg=-97C & Tm=320C	Autofluorescence & Focusing problems	

According to the set needs and specifications, thermoplastics are the best fit for our application, as a material to choose for the chassis, as thermosets have many limitations to use, manufacture, assemble and treat, metals are not transparent and can be hardly manufactured into small features, ceramics and glass are very brittle and thus are susceptible to cracking breaking and thus can hardly be fabricated into the needed microstructures. Thus, thermoplastics are best fit to our applications as a potential material for the chassis. Therefore, a list of the common thermoplastic materials available in the market and fit into the set needs and specifications (defined before), are compared according to the most vital mechanical, thermal, chemical and surface properties, in addition to the factors of price and availability in the market availability. For the mechanical properties, the tensile strength, and the tensile modulus of elasticity were

assessed. The potential material that is to be chosen should be a strong material, having a high modulus of elasticity (preferably >2500MPa), and a high tensile strength (preferably >65MPa), in order to be able to scaffold the membrane with its bending pressures with minimal deformations.

For the thermal properties of the membrane, the coefficient of linear thermal expansion, in addition to the glass transition temperature and the melting temperatures were assessed. The potential material should have a low coefficient of linear thermal expansion (preferably<2.5x10^-5/C) for the material not to be drastically deformed while heat treating. In addition, the material should have a glass transition temperature larger than the room temperature, to be able to preserve its rigidity (preferably > Tg of PET= 81 C), and a melting temperature larger than 150 C (preferably close to the Tm of PET = 264 C) to be flexible with the range of temperatures when heat treating or thermos-fusing the parts together.

For the chemical and biological properties, the water absorption rate and the ethanol resistance were assessed. The potential material to be selected should have a high-water absorption rate (preferably >0.2% per 24 hrs) in order for the adhesive to bind to it, and the gels to distribute in the wells when performing a biological experiment. In addition, the material should be ethanol resistant, since ethanol may be used to sterilize the device before using biologically.

Other parameters such as the price and the market availability are also taken into consideration while assessing the materials. The potential material to be selected should be cheap and available in the market.

The assessment process of the materials came as follows:

- Compare each of the properties of a specific material with that of the material having the optimal property (either having the maximum value or the minimum value) and get the ratio of the absolute value of the difference between it and the least desirable (either minimum value or maximum value) over that of the absolute value of the difference between that of the optimal from the least desirable.

$$Ratio = \frac{|\ Current\ value\ being\ assessed - least\ desirable\ |}{|\ Optimal\ value - least\ desirable\ value\ |}$$

For example: to assess the tensile strength of PMMA= 69 MPa and knowing that the optimal tensile strength is the maximum value, we compared it with that of the tensile strength of PEEK= 97 MPa, which is the maximum tensile strength in the list (optimal), and with that of PPO=0.01MPa, which is the minimum tensile strength in the list (least desirable). And then we calculated the ratio to be:

$$Ratio_{tensile\ strength,PMMA} = \frac{69-0.01}{97-0.01} = 0.7113\ (71.13\%\ of\ optimal)$$

Another example, assessing the coefficient of linear thermal expansion of PMMA=2.2 x10^-5/C, we compared it with that of the coefficient of linear thermal expansion of PEEK=1.4 x10^-5/C (optimal minimum value) and with that of LDPE= 16 x10^-5/C (least desirable maximal value).

$$Ratio_{coefficient\ .of\ \ linear\ thermal\ expansion,PMMA} = \frac{16-2.2}{16-1.4} = 0.9452\ (94.52\%\ of\ optimal)$$

For qualitative parameters such as ethanol resistance and market availability, we give relative values for each of the given values. For example, for ethanol resistance, if the value= poor then ratio = 0, if the value= good then ratio=0.5, and if the value=excellent

then ratio=1. Similar process is done for to the market availability, if the value=custom then ratio=0, if value= available then ratio=0.5, if value=ready then ratio=1.

- Give weights percentage (according to the importance of each property) to the ratios of each of the properties for each material, then add the ratios multiplied each by its weight, to get the overall applicability ratio for each of the materials. We set a weight of 6.25% for each of the ratios of Tg and Tm, and weights of 12.5% for the rest of the parameters ratios.

$$Overall\ applicability_{Material}$$
$$= 0.125x(Ratio_{tensile\ strength,Material}$$
$$+ Ratio_{tensile\ elastic\ modulus,Material}$$
$$+ Ratio_{coefficient\ .of\ \ linear\ thermal\ expansion,Material}$$
$$+ \ Ratio_{water\ absorption,Material} + Ratio_{ethanol\ resistance,Material}$$
$$+ \ Ratio_{price,Material} + Ratio_{market\ availability,Material})$$
$$+ 0.0625\ x\ (Ratio_{Tg,Material} + \ Ratio_{Tm,Material})$$

For example:

$$Overall\ applicability_{PMMA}$$

$$= 0.125x(Ratio_{tensile\ strength,PMMA} + Ratio_{tensile\ elastic\ modulus,PMMA}$$

$$+ Ratio_{coefficient\ .of\ \ linear\ thermal\ expansion,PMMA}$$

$$+ \ Ratio_{water\ absorption,PMMA} + Ratio_{ethanol\ resistance,PMMA}$$

$$+ \ Ratio_{price,PMMA} + Ratio_{market\ availability,PMMA})$$

$$+ 0.0625\ x\ (Ratio_{Tg,PMMA} + \ Ratio_{Tm,PMMA})$$

$$= \ 0.7\ (70\%\ of\ the\ optimal\ combination)$$

- Rank the materials according to their overall applicability values in descending order. Such that the material with the highest overall applicability ratio will have a rank of one, and that of the lowest overall applicability ratio will have a rank of eighteen (since we have 18 materials being studied)

Table 4 Chassis materials listing, analysis and selection

	Overall Rank	Overall applicability	PROPERTY	Tensile strength	Tensile elastic modulus	Coefficient of linear thermal expansion	Tg	Tm	Water absorption 24hrs	Ethanol resistance	Price	Market availability
		%of optimal	UNITS	Mpa	Mpa	10^-5 /C	C	C	%		$ / kg	
		100.00%	Optimal	12.50%	12.50%	12.50%	6.25%	6.25%	12.50%	12.50%	12.50%	12.50%
AMORPHOUS THERMOPLASTICS	17	38%	ABS	28.3	2,027.1	3.1	110	125	0.30	Poor	19.00	custom
	4	70%	PMMA	69.0	2,757.9	2.2	105	160	0.20	good	3.80	Ready
	15	48%	PETG	53.1	2,206.3	2.1	69	245	0.20	excelent	37.50	custom
	10	60%	PS	24.1	1861.6	2.5	100	242	0.1	excelent	3	available
	6	67%	PVC	51.7	2,833.8	1.8	81	220	0.06	excelent	2.50	available
	12	55%	PPO	0.01	2413.2	1.8	175	250	0.07	excelent	2.00	custom
	5	68%	PC	65.5	2,378.7	2.1	147	157	0.15	excelent	3.80	available
	8	64%	PSF	70.3	2482.1	1.7	190	520	0.30	good	26.00	available
SEMICRYSTALLINE THERMOPLASTICS	14	50%	HDPE	28	1,250	3.9	-125	135	0.1	excelent	4.00	available
	18	28%	LDPE	10	250	16.0	-130	125	0.10	good	2.00	available
	2	74%	NYLON	86	2,758	2.5	55	235	0.8	excelent	6	custom
	7	66%	PBT	60	2,868	4.0	22	250	0.08	excelent	2.80	available
	1	83%	PEEK	97	3,378	1.4	143	357	0.50	excelent	8	available
	3	71%	PET	79	2,758	2.2	81	264	0.10	excelent	2.00	available
	13	52%	PP	37.2	2000	8.0	-18	179	0.001	excelent	5	available
	9	63%	PPS	86	3,310	2.2	88	280	0.02	excelent	13.00	custom
	16	48%	PTFE	14	700	3.1	-97	320	0.001	excelent	7	available
	11	59%	PVDF	54	2,413	4.0	-30	176	0.02	excelent	6.00	available

rank	Material
1	PEEK
2	NYLON
3	PET
4	PMMA
5	PC
6	PVC
7	PBT
8	PSF
9	PPS
10	PS
11	PVDF
12	PPO
13	PP
14	PETG
15	HDPE
16	PTFE
17	ABS
18	LDPE

Results had shown that PEEK has the highest overall applicability ratio (83%), followed by Nylon (74%), PET (71%) and PMMA (70%). On the other hand, LDPE has the lowest overall applicability ratio (28%), followed by ABS (38%) and PTFE (48%). The decision goes by choosing PMMA rather than PEEK, although PEEK was optimal, since it was the only available material ready for use at the time, but in the future, we aim to use PEEK, PET or NYLON.

2. *Detailed Design Description and Parameters Needed to be Optimized*

After selecting the optimal materials for both the membrane and the chassis, we should now find the optimal design parameters dimensions, that depends mainly on the resemblance to the real tissue and the other main needs and specifications set before, in addition to additional limitations bounded by the materials chosen for the membrane (PET) and the chassis (PMMA).

a. Duct's Channel Diameter

One of the most important geometrical parameters of our design is the duct's channel diameter, since it is the basis of our main assumptions and hypothesis on the cellular expressions & interactions, and the epithelial cells buildup into a full duct. Choosing a diameter for the ducts of our LOC is not an easy task, as it depends on the resemblance to the real tissue, the fabrication techniques ability & material strength capacity to cut a small channel into the chosen PMMA chassis. And most importantly, the diameter depends on the ability of the membrane to deform into a circular cross-sectional hemi-channel without breaking or plastically deforming, leading in a drastic change in the pore size. For the resemblance to the real tissue, it depends mainly on the architecture of the real ductal tissue. The ductal system has a grape-like structure, with ducts having the largest diameter at the level of the nipple (around 2mm) and goes down in size as we

go deep through the tissue, until reaching its smallest diameter (around 20um) at the level of the acini. Invasive ductal carcinomas emerge in lactiferous duct that connects through a branched system, the nipple to the lobules (ranging from 0.1mm – 1 mm) and have an average diameter of 0.57mm.

- Specifications: the ductal diameter should resemble the real ductal diameter at the locations where invasive ductal carcinomas emerges (diameter=0.1 – 1mm). In addition, it should be suitable for the current fabrication techniques on PMMA, such as milling (common round milling tips sizes that fits in the above range=0.1,0.2,0.3,0.4,0.5,0.6,0.8,1 mm), molding (minimum flow channeling distance of PMMA), or UV cutting. Another important noting is that the wells depth also depends on the duct channel diameter, since we want them to be open to each other. Thus, the bottom layer thickness of the wells depends on the duct diameter, since the maximum thickness of the bottom well layer should be less than the radius of the cut duct channel. Note that geometrically, for an angle of 30 degrees from the centerline / axis of the depth of cut from hemi-duct groove, the thickness of the bottom well layer is equal to half the radius of cut, and the open area is 1.42 times the radius of cut, or 71% of the full duct diameter.
    - Methodology: The minimums membrane deformations are to be calculated analytically or computationally, to know the limitations of the ductal deformations, and thus know the minimum duct channel diameter that could be formed for our design using the chosen PET membrane. Computational simulations and finite element analysis were done using SolidWorks, to find the minimum Elastic deformation diameter a 10-um thick PET membrane could form, and the maximum strain rate at this value, giving us indication on the change in

the pore size on the deformed membrane. Additionally, Experimental iterations were done on the chassis manufacturing using CNC milling machine (HAAS VF-6 CNC Milling Machine (5 Axis)), to find the optimal cutting diameters using ball-mill tips. Ball-mill tips grooving (diameters = 0.1, 0.2, 0.3, 0.4, 0.5, 0.6, 0.8, 1 mm) are tried and assessed qualitatively according to process precision (errors in handling, positioning and referencing and cutting) and the cuts are measured using an electronic caliper, and the values having 5-6% maximum errors are only accepted.

- Results: The FEA simulations on the membrane deformation had shown that the minimum elastic deformation of the membrane into a circular-cross-sectional hemi-channel is 50um at a strain rate of 10%. For the Experimental iterations on the PMMA chassis, an average of 20 +/-10um error was observed, this is 10-30% error of the 0.1mm cut, 5-15% error of the 0.2mm cut, 3.3-10% error of the 0.3mm cut, 2.5-7.5% error of the 0.4mm cut and 2-6% error of the 0.5mm cut, 1.67-5% error of the 0.6mm cut, 1.25- 3.75% error of the 0.8 mm cut, and 1-3% error of the 1mm cut. Thus, the minimum cut diameter for a 6% maximum error is the 0.5mm diameter ball mill cut, in addition that it fits the average value of the resembled tissue.

b. Wells Diameter and Depth

Another important geometrical aspect of our design is the wells' dimensions, since it is the basis of the major limitation of our design which is the well's bottom wall thickness. Choosing the wells depth depends on the ability of the material to withstand the cutting forces and precision error, specifically to find the well's bottom wall thickness without breaking, melting or

deforming. In addition, choosing the wells depth depends also on the thickness of the PMMA sheet needed to be cut, which also depends on the maximum visualization depth using a confocal microscope (2mm), and the minimum thickness of PMMA found experimentally (1.8mm) where it can be held/positioned on the CNC machine firmly, without bending and creating more geometrical errors. Choosing the wells diameter depends on the ergonomics, minimum dispensing volume (0.5ul).

- Specifications: the well depth should be suitable for the current fabrication techniques on PMMA, such as end-milling, molding, or UV cutting. In addition, the wells depth depends on the duct channel diameter, since we want them to be open to each other. Thus, the bottom layer thickness of the wells depends on the duct diameter, since the maximum thickness of the bottom well layer should be less than the radius of the cut duct channel. Geometrically, for an angle of 30 degrees from the centerline of the depth of cut from above, the thickness of the bottom well layer is equal to half the radius of cut, which is 0.125um for a duct diameter of 0.5mm, and the open area is 1.42 times the radius of cut, or 71% of the full duct diameter, which is 0.355um for a duct diameter of 0.5mm. An open area to the well of at least 70% of the duct diameter is needed, but without breaking the wells bottom wall broken open. For the wells diameter, it should be large enough to be handled easily during biological experiments while dispensing the gels in it, with minimal risk of cross-contamination
- Methodology: The duct diameter is first assessed ergonomically, by three people with no prior experience in pipetting, where 4 plates each with 25 wells (that are then numbered) having diameters of either 1.5, 2, 3, 4 or 5mm at a proximity of 0.3mm from each other had been tried. The experiment goes by each of the three individuals (n=3) trying to

pipette 100um of stained water in the wells with two different colors, blue for the odd numbered wells and red for the even numbered wells, and repeating three times (three technical replicas), and the assessment goes by counting the number of wells that are cross-contaminated by two different colors. The duct depth (bottom wall thickness) is subjected to experimental testing, where 28 LOCs each containing 25 wells 3mm in diameter, are cut up until the bottom well thickness reaches 200um (1.8mm deep wells), 150um (1.85mm deep wells), 100um (1.9mm deep wells), 75um (1.925mm deep wells), 50um (1.95mm deep wells), 30um (1.97mm deep wells), 20um(1.98mm deep wells). And then the wells are assessed qualitatively for the number of wells that fails due to the loss or break of the wells bottom wall, for 28 different trials on the 7 different cases each repeated four times(n=4), and 25 wells were assessed per trial (technical replicas). Statistical analysis was done on the data where one-way ANOVA was used to test for the experimental significance and post-hoc Tukey was used for calculating the significance of individual parameters.

- Results: The pipetting experiments and the relation between the wells diameter and the ease in pipetting had shown that on average, mistakes of cross-contamination between the wells occurred significantly when pipetting in wells having diameters of 1.5mm (34%) and 2mm (18%) when compared to using wells having diameters of 3 (4%), 4 (2%) and 5mm (1%). Thus, the smallest well diameter that can be used for an ergonomically practical pipetting and handling is the 3mm diameter wells.

  Results of the end-milling experiments of the wells up to a predefined well bottom wall thicknesses, showed that the CNC milling process have a 20um +/-10um error. Thus, when milling the wells up to 1.98mm and 1.97mm keeping a well bottom wall of 20 um

and 30um respectively, 92% and 65% of the wells significantly failed respectively. While when milling the wells up to 1.95mm, 1.925mm, 1.9mm, 1.85mm and 1.8mm keeping a well bottom wall of 50um, 75um, 100um, 150um and 200um respectively, only 5%, 3%, 2%, 1% and 0% of the wells failed respectively, with no significant difference between the outcomes. Thus, the deepest well that we choose where the bottom wall didn't fail is the 1.95mm deep well having a bottom wall 50 um thick.

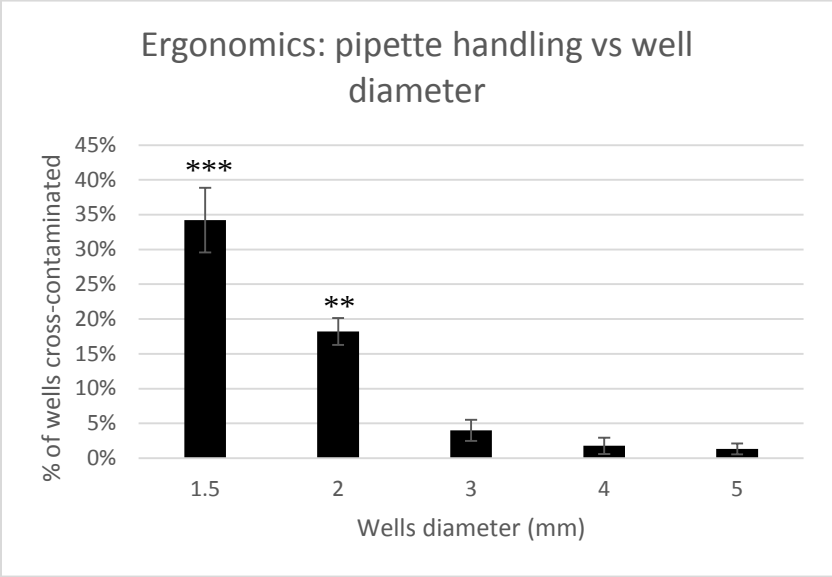


Figure 17 Ergonomic testing on the well diameter effect on pipetting and handling errors

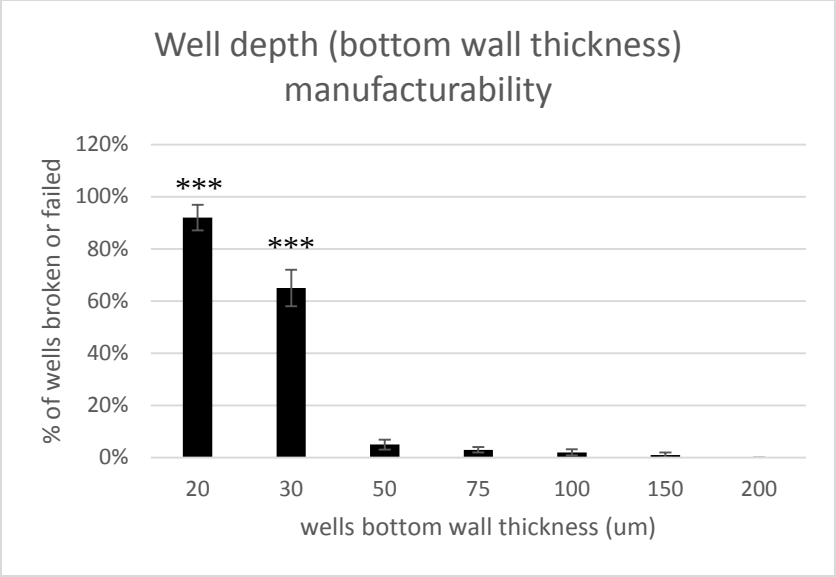


Figure 18 Well bottom failure test (minimal thicknesses).

c. Wells Proximity to Each Other

Another important geometrical aspect of our design is the wells proximity to each other, since the closer they are to each other, the less non-biomimetic areas we have (areas where cells are surrounded with plastic rather than stroma from the other side of the membrane). But having the wells closer to each other creates many other problems, most importantly the risk of breaking of the walls in between the wells and the non-homogeneous adherence of the membrane to the area in between the wells thus having the risk of cross-contamination between the wells from below between the wells and the membrane.

- Specifications: the wells proximity to each other should be suitable for the current fabrication techniques (milling, laser cutting or UV cutting) on PMMA without melting, breaking or deforming and with keeping a layer in between the wells which is thick enough for it not to break while handling.

- Methodology: The wells proximity to each other parameter was assessed by performing experimental testing on 75 PMMA plates each containing 25 wells (cut using Epilog Legend 36EXT laser cutting machine or milled using the HAAS VF-6 CNC Milling Machine (5 Axis) at AUB workshops), having diameters equal to 5mm, 4mm, 3mm, 2mm or 1.5mm and of each five different well proximity distances 0.3mm,0.2mm, 0.1mm, 0.075mm, 0.05mm, 0.025mm and each case had three replicas. Assessment was done by counting the wells that deformed or broke open to each other for each of the cases. Note that each plate contains 25 wells (5 horizontal x 5 vertical) thus have 40 overall areas of contact.
- Results: Laser cutting the well holes had shown that only the well proximity of 0.3mm resulted in no well proximity failure for all the 5 diameters tried. Thus, laser cutting is not the optimal way to create the wells since it has a precision of +/-0.1mm which is very high relative to our application. For this reason, the experiment was repeated by milling instead of laser cutting the well holes since the milling machine has a much more accurate process and better precision cutting. Results had shown that the optimal well proximity (which is the minimum proximity with no failure) for each well diameter was as follows in the below table:

Table 5 List of optimal wells proximity values for each corresponding well diameter.

Diameter	Optimal Proximity
5mm	0.3mm
4mm	0.2mm
3mm	0.1mm
2mm	0.075mm
1.5mm	0.075mm

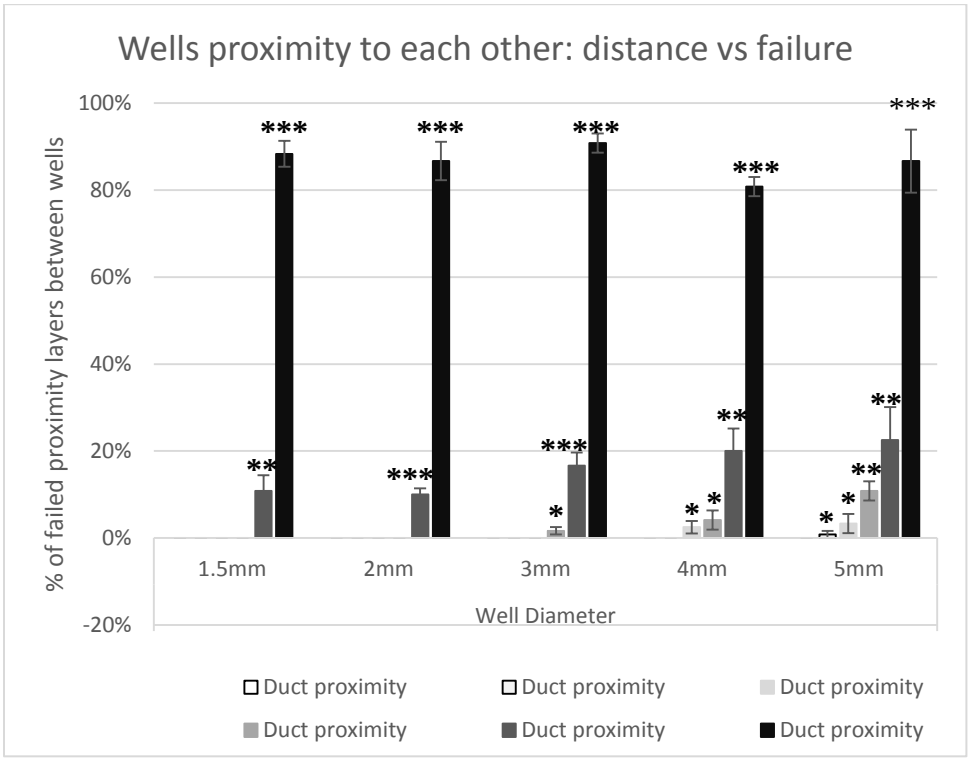


Figure 19 Wells proximity failure test for various well diameters

d. Inlet and Outlet Holes Dimensions and Designs

One of the major problems of microfluidics devices is the tubing connectors, since it requires either a larger hole depth to diameter ratio enabling the pressure fit of the tubes inside the holes, or it requires the usage of a connector as an intermediate step thus imposing either a bulk assembly.

- Specifications: the inlet and outlet holes diameter should fit to the standard microfluidic tubing found in the market. In addition, the tubing when connected to the inlet or outlet holes should not slip or disassemble while handling, thus requiring a hole depth to tube diameter ratio to be larger than 1 or requires a very stable connector step.

- Methodology: Drill 15 PMMA plates each containing 12 holes having diameters equal to either 1mm, 1.5mm, 1.58mm (1/16”), 2mm or 3.17mm (1/8”) (n=3 of each). Afterwards, PTFE, Tygon and stainless-steel tubes are then pressure fit (or threaded) in the holes. Then, bending tests were performed by 3 different people on the tubes connected to the holes, by applying a small handling force on the connected tube 10 times in the directions of right then left then front then back. The stage upon which the tube slipped or disassembled is recorded for each of the tube types, hole diameters and connector types. Noting that 10 rotations were done on tubes connected to each hole on 4 different directions, thus 40 tube movements were done, in addition that 12 holes/trials exist in each plate where the 4 different connectors are inserted, 3 of each type.
- Results: Bending experiments on the tubes connected to the holes has shown that threading the PMMA sheet that is only 2mm thick results in the thread wearing, especially when stainless steel tubes were used. For this reason, and knowing that tygon tubes can’t be threaded, only PTFE tubes were threaded, and experiment was performed on it, other tubes were press-fit into the holes. Results of the bending experiment on the effect of the diameter on the risk of slippage while handling had shown that all the tubes and holes having diameters of 2mm and 3.17mm (1/8”) had slipped after less than 17 movements. While tubes/holes having diameters of 1.5mm and 1/16” had shown to have similar fixation results which is more than 2x better than the 2mm and the 1/8” holes/tube, but most significantly the tygon tubes that showed few to no slippage for all of the trials. The 1mm holes/ tubes showed no slippage for all of the connection types, but they are not selected for the LOC application because they were custom made and not easily available in the market. Thus, we choose to go with the tygon tubes pressure fit in

the 1.5mm or 1/16” inlet & outlet holes for the PMMA LOC application. But an interesting observation on the experiments had shown that most of the firm connections failure (stainless steel, PTFE, and PTFE threaded) were because of the failure of the PMMA, and thus using another stronger material in the future such as PEEK or PET would solve this problem.

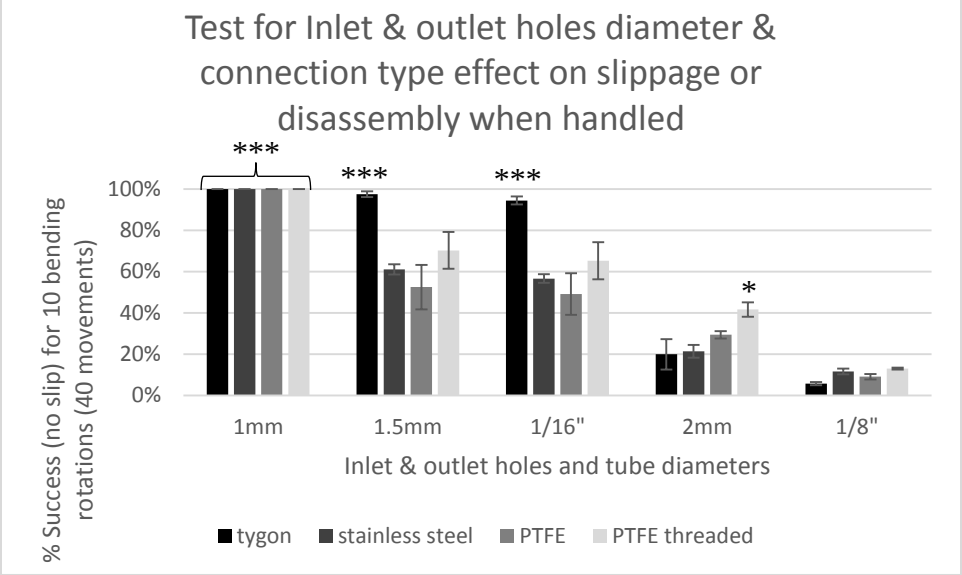


Figure 20 Ergonomic tests on the tube inlet & outlet diameter: tube slippage percentage of times

Figure 21 tube fitting and connection to LOC (a) pressure -fit tubing (b) threaded tubing PMMA chassis

e. Holder and Connections Design and Dimensions

The LOC device that we designed was aimed for optimal functionality according to the set needs and specifications, but one of the specifications was being user-friendly and adaptable to the current biological experimentation setups and techniques. One very important aspect to consider is the connectivity of the LOC with the microscope, with a waste chamber to be kept sterile and with a holder to assist in the handling of the LOC in biological experimentations without the risk of contamination.

- Specifications: The holder of the LOC should be an accessory to be connected when using the LOC. It should be able to firmly hold the LOC in position and without adding much to the thickness, so it wouldn't affect the pipetting process ease or the microscope visualization. In addition, the LOC holder should have dimensions suitable to its connectivity to the microscope and suitable for it to fit into a 10cm Petri-dish when needed in a biological experiment to relocate the LOC into a non-sterile environment (such as when visualizing cells under the microscope, or when moving the LOC from the BSL2 cabinet to the incubator where it'll stay for an extended period of time..

- Methodology: In order to solve this design problem, we should use the same iterative method that we used to develop the conceptual design of the LOC. In a way that we should first propose all the solutions applicable according the defined needs and specifications and based on the current solutions for similar problems and designs. And then we should select one of the designs we proposed, compare it with all the other designs and do analyze it accordingly, and then iterate and enhance the design and compare it with the others and then re-iterate until the optimal design was found.
- Results: The design that we chose based on the iterative method that we used on all the designs that we proposed, have external dimensions similar to that of the microscopic slide (2 plates 1mm thick and 75x25mm in length and width). In addition, the surface of each of the two cut plates used for the holder will be coplanar with the LOC surface when assembled in order for the microscope to take the surface of the holder (which is the default zero reference of the microscope) to be the same as the reference point for the surface of the LOC, thus easing the experimental process. The two plates are connected in a way that its always co-planar with the LOC surface not just from one side, but from both sides, so that it makes is easier for the user to make the maximal use of the microscopic facilities. The two plates of the microscope holder is separated by four fixtures located at the edges of the LOC to hold it firmly into the holder, in addition those fixtures have customized thicknesses, so that when assembled with the two holder plates the overall holder will have the same thickness as the LOC.

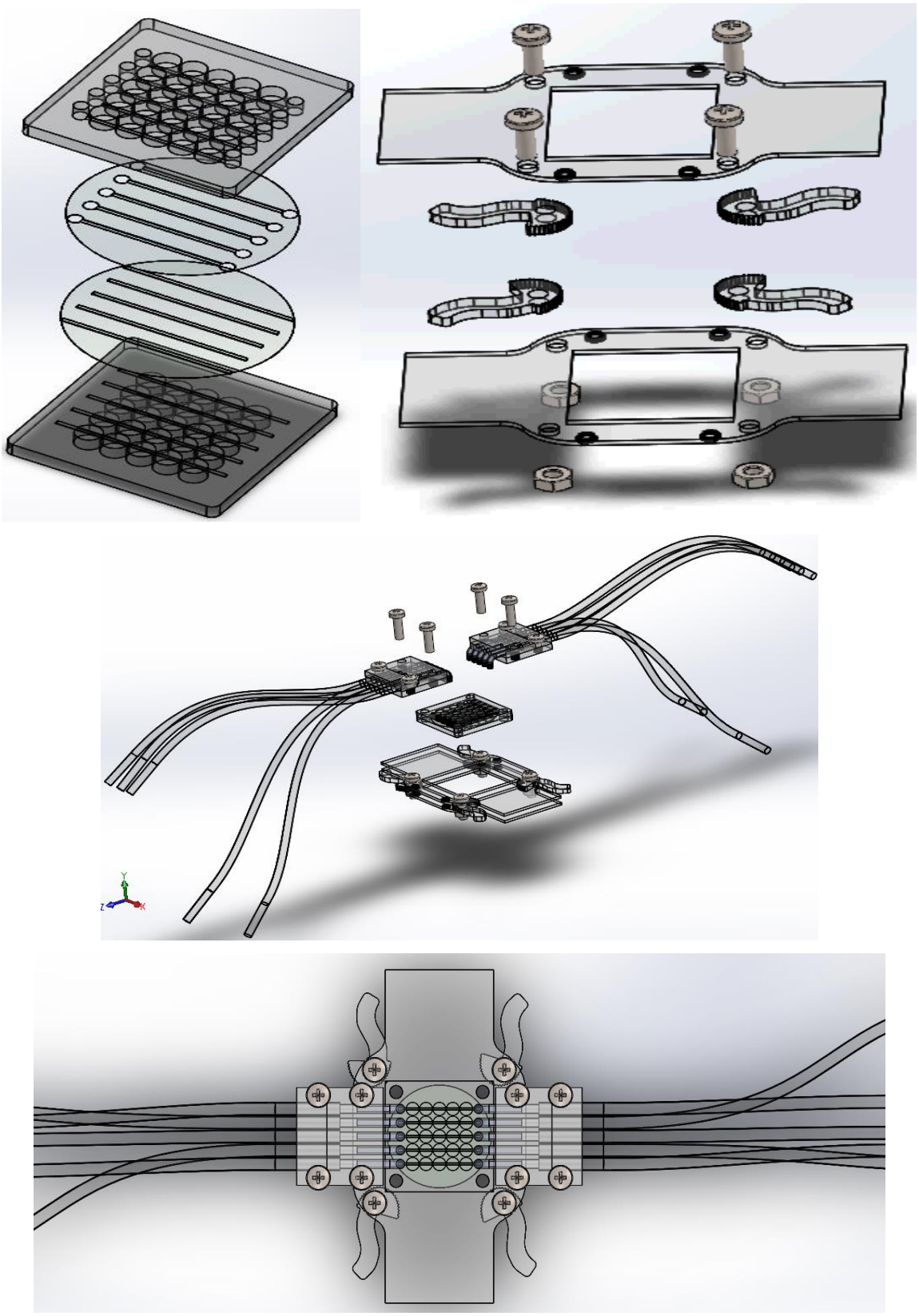


Figure 22 CAD drawing of LOC (a) LOC exploded view (b) holder exploded view (c) LOC connection to holder exploded view (d) LOC connected to holder and tubing top view

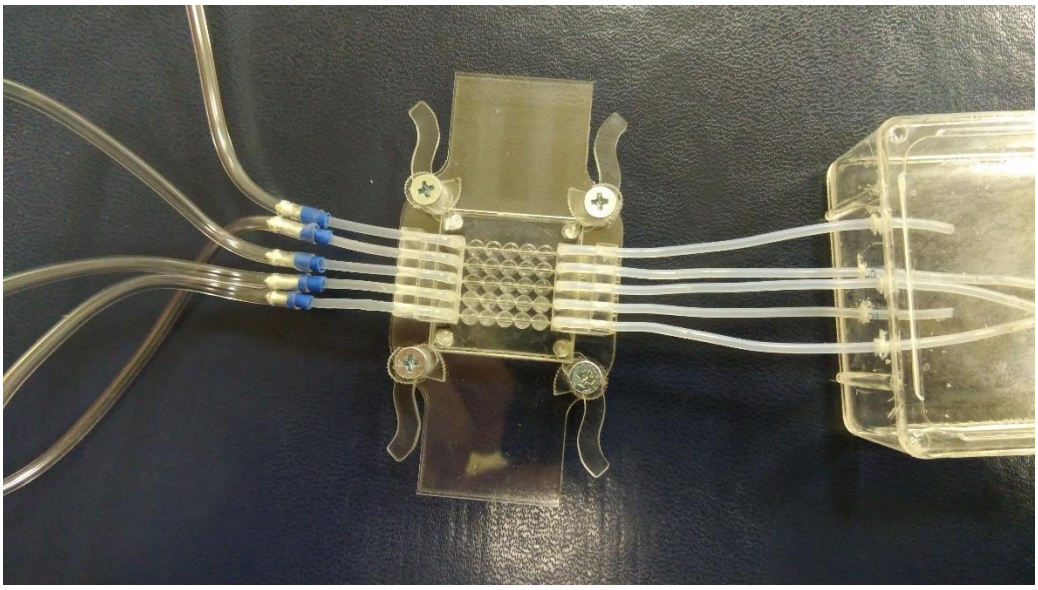


Figure 23 LOC connected to holder and tubings

C. Material Testing for Toxicity & Adhesion and Surface Modification

After selecting the materials to be used for the LOC according the set of predefined specifications indicated before, it is important to test the biological relevance of the materials selected, before taking it forward to the detailed design stage. It is important to note that materials bought from the market aren't always pure enough to sustain a biological experiment, since some minor modifications in the material or surface properties. The material could be modified either at the industrial stage or at the translocation stage being in contact with corrosive materials, pollutants or other modifiers that may affect the cellular interaction with the material.

1. *Culturing Cells on Top and in Proximity to Surfaces and Materials used in LOC Buildup and Visualize Cell Death and/or Adhesion*

As we discussed and validated in this section, our LOC is composed of two thin porous PET membranes and two PMMA chassis parts that are to be connected to each other either by welding or by an adhesive layer. Thus, we assessed the effect of all of the material used on the cell viability and adhesion on the PET membrane while being in proximity with the other materials used in the assembly of our LOC.

a. Specifications

All the materials used in our LOC fabrication and assembly should not be toxic to cells whether they are to be included in areas in contact with the cells or just in proximity to cells. In addition, the biological surface properties, particularly cell adhesion, is to be determined in addition to the effect of other materials used in our LOC as an adhesive layer for example on the cell adhesion to the surfaces.

b. Methodology

To assess the effect of materials on cells viability and adhesion, MDA-MB-231 cancerous breast epithelial cells are seeded on top of PET membranes (500,000 cells per membrane) in proximity to PMMA in one experiment and on top of membrane heated and then cooled by quenching or by slow cooling. And cells are also seeded on top of the PET membrane in proximity to PDMS (which may be used as an adherent layer) with different base: binder ratios (10:1 and 10:3), to assess if excess of the binder or base material may be toxic to cells. In addition, cells are also seeded on PET membranes in proximity to the same PDMS base binder ratios (10:1 and 10:3) but diluted in hexane (ratio of silicone: hexane is (1:2)) to check if the solvent will still be toxic after curing. The reason why the effect of PDMS surfaces on cell adhesion is assessed is because

it is important to note that when using an adhesive to assemble microfluidic devices, there is a high risk of leakage into the cell-culture channels, thus it is it important to note the effect to be able to characterize a leakage of silicone if it happens. To assess if the cell death occurs due to toxicity or just since cells couldn't adhere to the surface. The PET membrane will be half coated with the material to be tested and if the all the cells cultured on the membrane died, then the death is due to toxicity, and if only the coated area resulted in no living cells, then the death is due to cells not adhering on top of the coated surface.

c. Results

Results shows that both PET membranes and PMMA are good for cell culture applications while comparing the density of cells seeded on the membrane and the plastic versus that seeded on top of the 6-well plate polystyrene surface. Noting that no toxicity occurred, bur cells adhered al little better on the PET membrane than on top of the PMMA plastic and on top of the 6well plate surface (results are not shown). In addition, cells seeded in proximity to PDMS and PDMS diluted with hexane, didn't show any toxicity, thus neither PDMS is toxic in all its combinations and its dilutions with hexane. But cells seeded on top of the PDMS with all of the different combinations didn't bind on top of the surfaces, thus PDMS with all its combinations is inert to cells, preventing cell binding on top of it (results shown in table below).

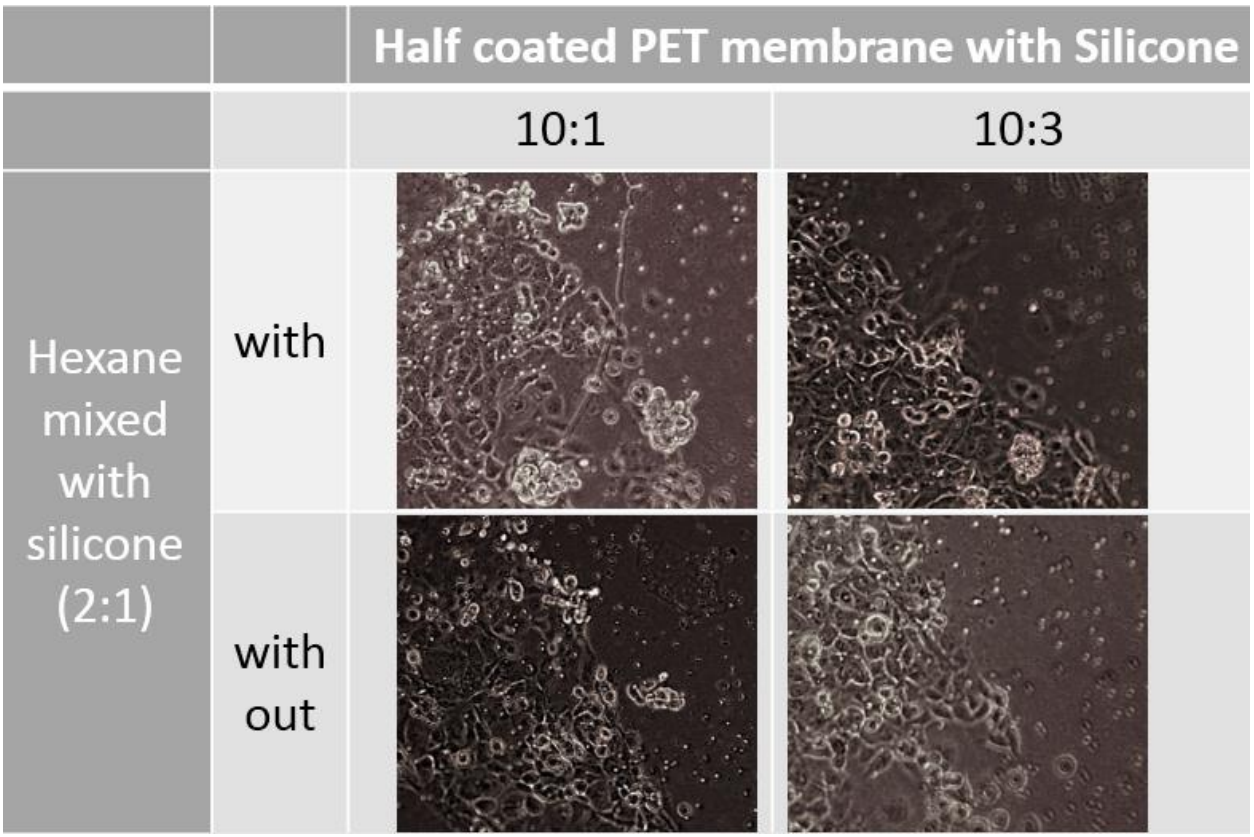


Figure 24 Effect of silicone concentrations and hexane dilutions on MDA-MB-231 breast cancer cells

2. *Coating Surfaces with Matrigel and Collagen with Different Concentrations and Optimize for Enhanced Adhesion and Viability*

In cases where leakage of silicone or any other inert or hydrophobic adhesive may happen, cells will not be able to adhere to the surfaces as shown above. But to assist the cell binding to the surface, the surface may be coated with collagen, Matrigel or any substance that may enhance the binding of the cells to PDMS. In this experiment we aim to test the effect of the coating of Matrigel, collagen, FBS (fibronectin) and poly-dopamine on the cell binding on PDMS coated surfaces, to check if the later may enhance the cell binding to the PDMS coated PET surfaces.

a. Specifications

All the materials tested should be applicable for usage in the LOC for future applications, in a way that no limitations should occur in terms of the ability applying the materials to the surface in terms of compatibility with the membrane material (PET), Ethanol or any material that may be used. The aimed material should be able to adhere to PDMS and cells on the other hand can bind to it.

b. Methodology

To assess the effect of the different coatings on the cell adhesion to the PET membranes coated with PDMS, we seeded MDA-MB-231 cells on top of the coatings (500000 cells per PET membrane), and we checked the cell density after 24 hours. We used 6 PET membranes, one was a control, two were coated with collagen of two different dilutions (1:50 and 1:100), one coated with Matrigel (1:30 in RPMI media) and one coated overnight with FBS media. Poly-dopamine was aimed to be used to enhance the binding of collagen to the PDMS but wasn't used due to the unavailability in the material at the time. The effect was assessed after 24 hours of seeding the cells on the surfaces.

c. Results

Results shows that Matrigel couldn't bind to PDMS, surface while collagen did enhance the binding of the cells to the PDMS coated PET membrane surface, but the cell density was significantly less than the control, thus further modifications in the experimental procedure is needed (concentration and dilutions modifications, the coating with poly-dopamine before adding the collagen among others). In addition, coating with media containing 10% FBS overnight enhanced the cell binding on top of the PDMS coated membrane but also not substantial compared to the control, thus, further enhancements should be done (coating with 10% FBS).

CHAPTER V.

DETAILED DESIGN PROCESS SELECTION AND OPTIMIZATION SPECIFICATIONS, CRITERIA, RESULTS AND PRODUCT TESTING

After choosing the materials needed to create our embodiment design according to the set specifications and needs, and after it had been subjected to a set of testing and validations on the mechanical, optical and biological properties of the materials, the design was now ready to proceed to the detailed design stage, where its fabrication process is to be optimized, and the LOC is to be tested accordingly. In the detailed design, a manufacturing and assembling process was chosen according to the design structure predefined in the conceptual design stage. In addition to being dependent on the materials and dimensions set in the embodiment design stage, and to the set of needs and specifications described before and modified and enhanced at each design stage. The fabrication process is made of two main processes, the manufacturing and assembling processes. The manufacturing and assembling processes are first chosen according to a set of needs and specifications, and then the manufacturing parameters are optimized after performing a set of experimental iterations or a set of analytical calculations. Afterwards, the assembling process parameters are optimized and then treatments and surface and materials modifications are then chosen accordingly in order to enhance the binding of the different components together. After optimizing the manufacturing, assembling and treatment processes according to an iterative methodology, leakage testing was done on the assembled LOC to check for any assembling errors, and accordingly, either more modifications and treatments will be done on the assembling process, or the biological experimentations will be carried on. If after testing for leakage and iterating through all the assembling and treatment methods the problem persists, then we should go back to the embodiment design stage, select another material, modify

and optimize the manufacturing process accordingly, and then select and optimize another assembling process and test for leakage then iterate for better material, surface or adhesive treatments for better adhesion and more precision. After testing for leakage and result in successful outcomes, biological experiments are performed on the LOC where cells are seeded on different coatings of the LOC materials, and the effect of different washing times on cells was found in order to find the needed parameters to design an experimental procedure for which further biological experiments would be done accordingly. Finally, cell viability and adhesion were tested on the LOC walls according to the designed experimental procedure.

A. **Fabrication Process Optimization**

As indicated before, the fabrication process is based on two main processes, the manufacturing process and the assembling process and treatments. The manufacturing process was chosen according to a set of needs and specifications and to preselected materials and design dimensions defined in the embodiment design stage. The assembling process was selected also according to design dimensions and the materials selected in the embodiment design stage, in addition to a modified set of needs and specifications depending on the connective layer properties in terms of strength, thickness and homogeneity.

1. *Manufacturing Process Optimization*

The manufacturing process of the chassis depends on the method that could give the highest precision, and the smallest cut features while still being able to perform the 3D profiling of the detailed designed featured. Our design is composed of interconnected features from both sides of the chassis. From one side, the grooves of the circular cross-sectional hemi-duct are to be

formed, and from the other side the wells are to be cut precisely until a flat bottom surface of the well is formed and is opened to the duct at the area of the cut grooves only. And in one of every two chassis parts manufactured, an additional feature is formed, which is the inlet and outlet holes, and is usually performed as a third step after grooving the ducts, flipping to the other side of the plate and milling the wells, and then on the same side of the plate the inlet and outlet holes are to be drilled. The manufactured process to be chosen should be able to form all of those cut features. We have three main potential processes which are, milling, laser cutting, UV cutting and molding, and all those processes are applicable on PMMA. In order to choose a specific manufacturing method, we should check the limitations of each of those processes. To start, the laser cutting was first assessed and tried, and the stated features couldn't be formed using it since, it has a low-cut precision and resolution (+/-0.1mm) which was huge when compared to the feature dimensions in our design (0.5mm-3mm cuts, 0.1mm proximity areas and 0.05mm layers). In addition, laser cutting can hardly do 3D profiling, specifically on the circular cross-sectional cut needed to form the hemi-duct groove. But an important step could be made after accounting to the errors of the laser cuts, which is the cutting of the chassis plastic from the outside into its external dimensions. UV cutting have a much higher precision than UV cutting and even the other manufacturing methods, but the problem is that similar to laser cutting, UV cutting cannot perform 3D profiling in the LOC, and thus cannot create the circular cross-sectional cut needed to form the hemi-duct groove. Molding was also assessed, it is the optimal way to fabricate the LOC especially at the long run for mass production, but the problem was in the mold itself, since first, at the prototyping stages, it is very expensive to create a mold for every LOC with constant enhanced features, and second, it is very hard to create this mold itself from a material that could be used as a mold for PMMA. The problem with the mold creation

was in the 3D profiling of the tiny 3D features of the mold, especially the 0.1mm cut of what will become the mold for the well proximity feature, and the 0.5mm diameter formation of the hemi-duct extrude that will mold for the duct. In addition to many more complexities, especially if the mold cutting process was manipulated by assembling very tiny components manufactured each on its own and assembled into a mold for the LOC. For this reason, the molding process of the LOC was ignored in the meantime and left for later stages where all the LOC design, material selection, manufacturing process, assembling process, leakage testing and biological testing was optimized after an extended set of iterations. The last manufacturing method is CNC milling, the precision of this method depends on the machine used, and the machine found at AUB has a resolution of 0.003mm /step resolution, which is good enough for our application. In addition, the CNC milling process, although complex, may be performed on more than one set of operations, each on one side of the chassis PMMA plate, requiring the referencing and zeroing each time the plate is relocated on the CNC holder.

a. Laser cutting parameters (laser power and cut speed)

The laser cutting process was selected to cut the PMMA chassis into its external dimensions, before being relocated into the CNC machine for further cutting of its detailed features. The laser cutting process is a fast and easy process but have low precision (+/-0.1mm error) and this error is susceptible to increase according to two main parameters of laser cutting, the laser power and the cutting speed. Optimizing those two parameters leads to a better cutting precision and thus less positioning and referencing complexities when performing the CNC milling process.

i. Specifications

To begin with the laser cutting optimization process, we shall define the needs and specifications on which process will follow accordingly. We should find the PMMA chassis laser cutting

power and speed for the maximum precision, smoothest cut and minimum melting errors. Thus, we should find the optimal laser cutting power and speed needed to cut exactly into a 2mm thick PMMA plate without excess power or time of cut (slow speed), in order not to melt excess material on the boundaries of the cut, leading to more errors.

ii. Methodology

The optimal laser cutting parameters into a 2mm thick PMMA was calculated analytically and then validated experimentally, since the PMMA used may not be pure and may contain many impurities, in addition to possible precision problems with the laser cutting machine.

- Analytical

The power equation upon which laser cutting functions, depends on the latent heat of the material, the cutting depth, the cutting diameter, the boiling point of the cut material and the latent heat of vaporization of the material.

$$P = \left(\frac{\pi}{4}\right) z\ v\ \rho\ d(CT_V + L_V)$$

$z = Depth\ of\ cut$

$v = Speed\ of\ cut$

$\rho = Density\ of\ material$

$d = Cutting\ diameter$

$C = Latent\ heat$

$L_V = Latent\ heat\ of\ vaporization$

$T_V = Boiling\ point\ of\ the\ material$

In this problem, we have all the properties of the material and the diameter of cut from the laser cutting machine properties. So, from that the ratio of the laser cutting power from that of the

speed of cut which is needed to perfectly cut our material can be found. And afterwards, different combinations of the laser power and the laser speed of cut are chosen for experimental iteration and optimization of the quality of cut in terms of precision and melting of the boundaries of the cut. Since, the analytical calculations are based on the ideal properties of the material and cutting parameters, no further analytical calculations on the laser cutting will be carried on, instead experimental optimization will be carried on based on the set of needs and specification of laser cutting.

- Experimental

To validate the analytical data and account to many invalidated parameters such as the cutting diameter, we performed experimental testing in which different combination of powers and speeds of cut are used, and then the overall reduction in the thickness of the intended cut dimensions was measured using an electric caliper of resolution (+/-10um). Laser powers of 10, 30, 50, 70 and 90 W are tried each with different cutting speeds of 10, 30, 50, 70 and 90 mm/min, and then the cutting feasibility and the cutting precision were measured. The cut 2mm thick PMMA sheet was aimed for L x W of 2.5 x 2.5 mm, but will result in a bit smaller dimensions due to the diameter of the laser beam spot, and due to the power density that results in an even larger errors and could be minimized by optimizing the laser cutting speed and power.

iii. Results

Results are assessed analytically by calculating the possible range of use of the laser cutting power and speed, in addition to being assessed experimentally by trying for a wide range of powers and speeds and assessing for the minimal cutting errors and maximum precision.

- Analytical

  $z = Depth\ of\ cut = 2mm$

$v = Speed\ of\ cut$

$\rho = Density\ of\ material = 1.18\ \ g/cm^3 = 0.00118\ \ g/mm^3$

$d = Cutting\ diameter = \sim 0.0762\ mm\ or\ less\ according\ to\ the\ machine\ specs.$

$C = Latent\ heat = 0.35\ \ cal/gK$

$L_V = Latent\ heat\ of\ vaporization = 130\ \ cal/g$

$T_V = Boiling\ point\ of\ the\ material = 374\ K$

$$P = \left(\frac{\pi}{4}\right) z\ v\ \rho\ d(CT_V + \ L_V)$$

$$\frac{P}{v} = \left(\frac{\pi}{4}\right) z\ \rho\ d(CT_V + \ L_V)$$

$\frac{P}{v} = \left(\frac{\pi}{4}\right) 2mm\ x0.00118\ g/mm^3\ x\ 0.0762mm(0.35\ \ cal/gK\ x374\ K + \ 130\ \ cal/g) =$

$0.03685032\ cal/mm = 154.0979\ \ W/(mm/s) = \ 2.56829\ \ W/(mm/min).$

Table 6 Analytical calculations of the optimal laser cutting parameters on a 2mm PMMA sheet

P(W)	10	30	50	70	90
V(mm/min)	3.9	11.7	19.5	27.26	35.04

- Experimental

Results of the experimental testing on the laser cutting power and speeds on the 2mm thick PMMA sheet can be characterized in four categories and the combination of two main effects which are the excess cutting (melting) of the PMMA and the homogeneity of the cut across different areas. For powers less than 50 W and velocities less than 30 mm/min (P<50W &

v<30mm/min), the resulted cut had an error larger than 0.2mm, but the cut was almost homogeneous across all the area of cut. For powers larger than 50 W and velocities larger than 30 mm/min (P>50W & v>30mm/min), the resulted cut had an error smaller than 0.2mm and a bit larger than 0.1mm, but the cut wasn't homogeneous across all the area of cut with areas having errors fluctuating widely between 0.1 and 0.2 mm. For powers larger than 50 W and velocities slower than 30 mm/min (P>50W & v<30mm/min), the resulted cut had an error much larger than 0.2mm (depending on excessiveness of the power and the minimality of the speed), in addition, the cut wasn't homogeneous across all the area with fluctuations of +/-0.1mm For powers less than 50 W and velocities faster than 30 mm/min (P<50W & v>30mm/min), the resulted cut couldn't cut through the whole 2mm thick PMMA. For this reason, the laser beam of power = 50W & speed= 30 mm/min was chosen, and the error of 0.1mm was accounted while cutting. (ex: when aiming to cut a 25 x 25 mm PMMA 2mm thick, account for a 25.1x25.1 mm size)

Table 7 Laser cutting speed and power ranges and their effect on cut quality

		Power	
		P< 50 W	P>50 W
Velocity	V< 30mm/min	Error > 0.2mm but homogeneous cut	Error >> 0.2mm & non-homogeneous cut
	V>30mm/min	Material is not cut to full depth	0.1mm <Error < 0.2mm non-homogeneous cut

b. CNC milling parameters

The CNC milling machine was chosen as it has a very high precision while can 3D profiling be performed on the features fabricated. The process of 3D fabricating the LOC using the CNC machine is complicated due to the small size of the LOC and the aimed cut features, in addition

to the positioning and referencing problems of the LOC on the CNC machine holder and the series of accumulating errors due to the relatively low precision of the laser cutting. The precision of the features created using CNC milling depends on two main CNC cutting parameters in addition to a set of handling precision requirements, mainly accounting to the laser cutting errors using the CNC machine, the proper and precise positioning perpendicular to the CNC milling tip and the barely apply pressure on the PMMA plate when connecting to LOC to prevent bending. After accounting to all the handling errors, another error persists which is either the dislocation of the PMMA plate while being cut, or the over cut or melt of the milled features (although air coolant was applied at all times). For this reason, the milling spindle speed and feed rate should be accounted for, to optimize the cutting process and feature precision.

i. Specifications

We should find the optimal milling feed rate and spindle speed that wouldn't result in the dislocation problems of the loosely held PMMA plate being cut, and without the need to secure the holding more and bend the PMMA plate surface leading to inhomogeneous cuts. In addition for the selected spindle speeds and feed rates, no melting or deformations or surface roughening should occur due to the increase in temperatures resulting from high cutting speeds.

ii. Methodology

Experimental testings were performed on the PMMA sheets in order to create our aimed cut features in the PMMA, where various spindle speeds of 2000, 3000, 4000, 5000, 6000, 7000, 8000, 9000, 10000, 11000 and 12000 RPM were tried each at different feed rates of 10, 20, 30, 40, 50, 60, 70, 80, 90, 100, 150 and 200 mm/min. and then the results are assessed qualitatively for the cases where dislocation of the PMMA plate occurs, cases where melting or feature loss occur. If many cases result in neither melting, feature deformation nor dislocation then the cases

were to be compared visually for transparency change and roughness, and the more transparent and smoother resulting process should be chosen.

iii. Results

After performing all the 132 different trials, and assessing them qualitatively, the spindle speed of 7000 RPM and feed rate of 100mm/min was chosen due to dislocation and feature deformation problems. Results had shown that for slow feed rates and spindle speeds (Feed rate <100mm/min & Spindle speed <7000RPM), minor vibration problems occur and resulted in either the dislocation or the misalignment of the PMMA plate being cut or the breaking or loss of some of the very delicate features of the design. For high feed rates and spindle speeds (Feed rate >100mm/min & Spindle speed >7000RPM), minor melting problems and feature deformations occurs and resulted in the loss of some of the very delicate features of the design. For slow feed rates and high spindle speeds (Feed rate <100mm/min & Spindle speed >7000RPM), major melting problems occurs and resulted in the loss of most of the delicate features of the PMMA plate. For high feed rates and low spindle speeds (Feed rate >100mm/min & Spindle speed <7000RPM), major vibration and dislocation problems occurs and resulted in the loss of most of the delicate features of the PMMA plate. All of the combination of the spindle speeds of 6000, 7000, and 8000 RPM and the feed rates of 90, 100 and 120 mm/min resulted in few to no feature deformations and dislocations, but quality check on the optical and roughness properties of the milled cuts lead to the 7000 RPM and 100 mm/min combination as the one being chosen.

Table 8. CNC milling spindle cutting speed and feed rates effect on feature quality & preservation

		Feed Rate	
		Slow (<100mm/min)	**Fast (>100mm/min)**
Spindle speed	**Slow (<7000 RPM)**	Minor Misalignments & Vibrations	Well's bottom breakage & major dislocation & feature loss
	Fast (>7000 RPM)	Duct deformation & major feature loss due to melting	Minor feature loss due to melting

2. *Assembling Process Selection*

As stated and explained in the design sections, our design is made of two porous PET membranes and two PMMA chassis, and thus we have two major assembly steps, first the PET with the PMMA chassis and then connecting the two subassemblies together by binding the two PET membranes together. Knowing that PET and PMMA are two different materials creates many option limitations since those two materials have widely different set of parameters. And knowing that the full ductal structure was created due to the assembly of the two PET membrane together, each forming a hemi-duct, also set many limitations, since no deformation, contamination, or substance leakage should occur from the binding interface of the two PET membranes and the formed full ducts. Thus, each of the two interfaces binding processes was assessed and an assembling process was selected accordingly.

a. Assembling process for PET membrane to PMMA chassis (PET-PMMA)

For the PET membrane and the PMMA chassis assembly, the assembling processes were limited to only adhesive materials / substances, since those components are made of different sub-

materials and have a wide spread of parameter ranges and properties. The different adhesives and assembling processes were compared and assessed according to a set of needs and specifications and various experimental tests.

i. Specifications

The chosen adhesive should be able to bind to both, the semi-crystalline PET membrane and the amorphous PMMA chassis. In addition, the adhesive layer should result in a minimum connective layer thickness and in the same time result in minimum membrane deformation and maximal homogeneous alignment and planarity of the membrane surface on top of the PMMA surface.

ii. Methodology

In order to assess the different adhesives and choose one accordingly, the different adhesives were applied, and the sub assembly was assessed qualitatively and quantitatively. Different types of acrylics, epoxies, cyanoacrylates and silicones were tried the thickness of the subassembly was measured using an electronic caliper (resolution: +/- 10um) to assess the significant change in thicknesses due to a relatively thick adhesive layer. In addition, the membrane was assessed qualitatively for the homogeneity of the adhesion that can be visualized from the planarity of the assembled membrane (all the areas except the ones deformed into hemi-ducts) on top of the PMMA surface.

iii. Results

Quantitative assessments of the different adhesives showed that for adhesives of high viscosity, regardless to the type of adhesive, had resulted in a thick adhesion layer (up to 100um), and in most of the cases lead to the expansion to other unintended areas such as the outside surface of the deformed PET membrane duct, leading to the clogging of the pores from the outside. Fewer

volumes of the viscous adhesives are then dispensed on separated areas and were tried on the surfaces and the quantities were optimized to expand exactly to the intended areas, but unfortunately this resulted in a low-quality adhesion due to the inhomogeneity and non-planarity of the assembled surfaces. Lower viscosity adhesives were tried and assessed and resulted in a much lower adhesive interface thickness (can't be measured using the caliper 10+/-10um) but resulted in a non-uniform binding to the surface, since different areas were more in contact to each other initially, and since the adhesive layer was initially thin, it couldn't bind the two layers together homogeneously. For this reason, an adhesive with a sacrificial solvent was proposed in which initially a thick adhesion layer is to be applied, thus connecting all the interfacial areas together homogeneously, but after treating, the sacrificial solvent will evaporate, and the adhesion layer thickness would decrease accordingly. Thus, silicone adhesive was selected for this process, since although it resulted in a thick adhesion layer when used alone, but it resulted in a thin uniform binding layer when using hexane as a sacrificial solvent. The problem is that in both cases (using hexane or silicone alone), the silicone didn't bind well to each of the surfaces, and thus surface treatments such as plasma, silane, heat treatment, lamination and different silicone base to binder and silicone to hexane ratios was required to enhance the surfaces binding to the PDMS silicone.

b. Assembling process for two PET membranes together (PET-PET)

To bind the two PET membranes together, much more options were included compared to binding the PET membrane to the PMMA chassis, the reason is that the two membranes are made of the same materials. But still, the binding process is subjected to many other limitations, most prominently is the fact that the membranes are very thin and pre-bound to the PMMA while being connected and bound together, and thus any assembling process should not interfere with

the PMMA material properties. Another very important limitation is the interface between the areas to be adhered and the aimed ducts to be formed by connecting the two sub-assemblies together, each forming a hemi-duct.

i. Specifications

The chosen binding process should be able to homogeneously bind the two semi-crystalline PET membrane surfaces together, with the maximum binding force and minimum layer thickness. But, the process done should be ensuring no leakage into the duct and minimum membrane deformations and non-planarity.

ii. Methodology

Since the materials are similar, then welding processes could be tried in addition to adhesives. Thus, to assess which process is to be chosen, we should try connecting the sub-assemblies at the PET-PET membrane interface using heat fusion welding at high Tg of the PET. In addition, different adhesives such as epoxies, acrylics, cyanoacrylates and silicones should be tried on the PET surface interface, and the low viscosity adhesives and sacrificial solvents should also be tried. The adhesion thickness was assessed quantitatively by measuring the overall added thickness using an electronic caliper (resolution +/-10um) and qualitatively, by assessing the membrane uniformity and the adhesive distribution and leakage into the duct visually or under the microscope.

iii. Results

Quantitative assessments of the different adhesives showed results similar to that of the PET-PMMA connectivity, in terms of high viscosity leading to a high adhesive thickness (up to 100um) and leading to leakage into the ducts in most cases which is very problematic. And adhesives have low viscosities resulting in non-uniform binding of the two surfaces, so that when

a liquid is to be passed inside the ducts, interdental leakage is most likely to occur due to the un adherence to many of the interference areas. Heat fusion was tried an worked only for dew cases which are at high glass transition temperatures near the melting temperature of PET (Tg < but near Tm) which was in most of the cases at temperatures higher than the melting pint of PMMA, which was also problematic. Thus, the optimal adhering method to be chosen is either by using a strong low viscosity adhesive that could bind to PET strongly and perform several treatments to ensure uniform distribution of the adhesive occurs throughout the interfacing surfaces, or by changing the chassis material into another material (preferably PET), that have a higher melting temperature, so that heat fusion welding will be applicable.

3. *Assembling Process Parameters and Treatments Optimization*

After selecting the assembling processes to be used to connect the different components of the LOC together, the optimization of those processes is required to get the maximum adherence force with minimal complications and errors. In addition, different surface, material and process treatments should be done to facilitate and enhance the binding and assembling process. At first the silicone preparation ratios should be optimized for maximal binding force and strength, and then the optimal silicone dilution in hexane should be found to get the maximum adherence but with maximal reduction in the layer thickness. Afterwards different heat treatments were tried and optimized in order to give the optimal conditions for the silicones to cure and for the sacrificial solvent (hexane) to evaporate while making use of the material properties at their glass transition temperature to enhance the binding of the surfaces with the silicones. Afterwards, the effect of lamination and preloading was assessed to find the optimal load value that should be applied on the two assembling layers to enhance the surfaces contact with the adhesive and with each other homogeneously. Then the effect of the heat treatments on the membrane was assessed

by performing SEM (scanning electron microscopy) imaging on the PET membrane porosity and microscopic structure for different cooling methods. Then, O2 plasma treatment was tried and assessed on the PET and PMMA surfaces to enhance the binding of the silicone to it, in addition that Amino-silane and epoxy-silane surface coating was suggested and planned for further enhancement of the binding of both the PET membrane and the PMMA chassis to the PDMS adhesive layer.

a. Silicone preparation ratios

A standard use of the PDMS silicone (Sylgard 184) was in 10:1 ratios, but previous experimental observations during the assessment of the assembling process selection had shown that different combinations of the base and binder of the PDMS silicone resulted in different mechanical properties of the resulting material. Thus, we hypothesized that maybe a different base to binder ratio combination will result in a stronger and a more adherent adhesive layer. An additional set of needs and specifications were set accordingly for the aimed properties of the silicone, and then a qualitative and quantitative method were used to assess the strength and the binding effects of different ratios of PDMS and then one of them was selected.

i. Specifications

The optimal adhesive aimed from manipulating the different ratios of the PDMS silicone base to binder ratios should result in a stronger adhesion layer. In addition, the aimed adhesive should result in maximum adhesion to both the PET and PMMA surfaces with minimal layer thickness, a confinement volume and a controllable spreading along wettable areas.

ii. Methodology

The effect of the PDMS silicones having different base to binder ratios, are to be first assessed qualitatively to check if there is a significant difference between the different ratios on the layers

adhesion forces and qualities, in term of membrane deformation and non-planarity. Afterwards the two most significant ratios were chosen to be assessed quantitatively for strength and stiffness.

- Qualitative:

For the qualitative assessment of the effect of the base to binder ratios of the PDMS silicone, various PDMS base to binder ratios were tried (20:1, 10:1, 10:3 and 2:1) and assessed for the binding capability to the PET membrane on top of the PMMA chassis layer, for minimum membrane deformations and maximum adhesive distribution and binding force.

- Quantitative:

To assess the effect of the base to binder ratio on the adhesive layer strength, different base binder ratios (10:1 and 10:3) were chosen and quantitatively assessed using the mechanical loading and measurement Instron machine, where tensile strain was applied, and the tensile stress was measured on 6 different specimens, three replicas to each condition. The specimen was a bone-shaped having dimensions of (2.5 x 5 x 50 mm) according to the ASTM standards, and the tensile rate applied was equal to 1mm/min for 20 min, and the stress strain curve was assessed

iii. Results

After performing the qualitative testing on the PDMS silicones with different base to binder ratios, the data was assessed visually for the homogeneity of the adhesive distribution and qualitatively comparing the binding force and the binding force distribution along the full surface interface. And then the best PDMS ratio was chosen along with the standard 10:1 PDMS ratio and was subjected to a quantitative tensile loading and measurement testing, to obtain the stress-

strain curve and get from it the modulus of elasticity and the ultimate strength of each of the materials tested.

- Qualitative:

For the qualitative test, the 20:1 base to binder ratio resulted in a lower stiffness material with excess non-cured material and a weak binding force, but a homogeneously distributed adhesive layer at the surfaces interface. While the 2:1 base to binder ratio resulted in a very stiff strong adhesive layer, but which is of non-homogeneously distributed thicknesses and strength along the material binding interface. On the other hand, both 10:1 and 10:3 resulted in a well distributed homogeneous binding interface with good binding forces. It should be noted that the 10:3 ratio resulted in a bit stronger and clearer surface interface, and that both didn't have good binding affinity to the PET and PMMA surfaces and thus further surface modifications is required.

- Quantitative:

For the quantitative test on the mechanical properties of the PDMS with different base to binder ratios, the stress strain curves of both materials are measured and the results shows that PDMS 10:3 has a higher modulus of elasticity (E= 2.4 MPa) and ultimate strength (1.2 MPa) when compared to PDMS 10:1 which has a lower modulus of elasticity (E= 1.9 MPA) and ultimate strength (0.8 MPa). Thus, PDMS 10:3 will be used further on.

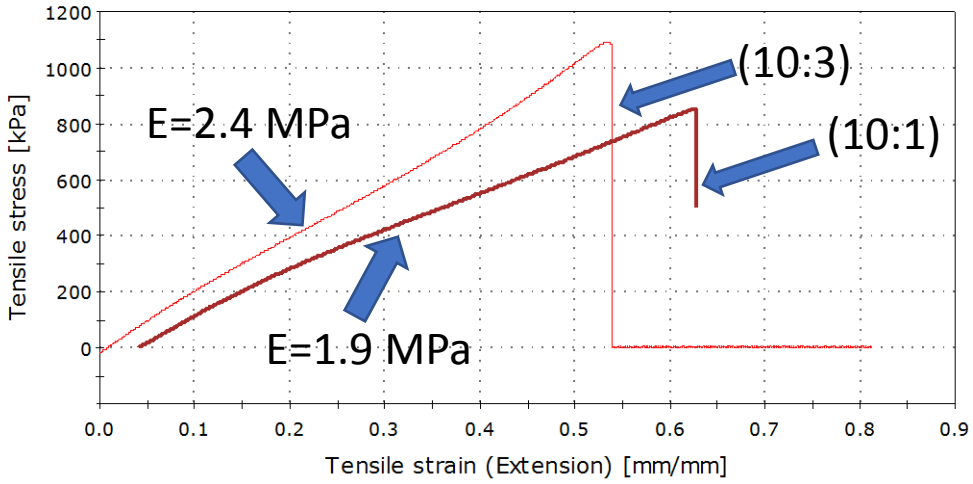


Figure 25 Stress strain curves of tensile loading on silicone with base : binder ratios of 10:1 & 10:3

b. Silicone dilution in hexane ratios

Hexane is a common solvent used with the PDMS silicone (Sylgard 184), but it was noted from previous experimental observations during the assessment of the assembling process selection that hexane, not only dilute the PDMS leading to a less stiff and thinner layer after the curing and evaporation of hexane, but also results in pulling the PET membrane towards the PMMA chassis in the process. This pulling force resulted in different effects when using the different PDMS base to binder ratios and different silicone to hexane ratios. A set of needs and specifications was set to define the properties needed to get the optimal use from this feature to assemble the LOC components together, and the different combinations were optimized after a set of qualitative and quantitative experiments described below.

i. Specifications

The optimal adhesive aimed from manipulating the different ratios of the PDMS to hexane should result in a strong adhesion layer. In addition, the aimed adhesive should result in

maximum adhesion to both the PET and PMMA surfaces with minimal layer thickness, should have an optimal viscosity to easily spread it, and should not leak to non-aimed areas and especially into the duct, if applied to bond the two PET membranes together. In addition, the resulted adhesive should have a relatively high compressive modulus of elasticity since it will be subjected to various treatments, namely heat treatment, lamination and weight loading.

ii. Methodology

To assess the effect of different combinations of the PDMS silicone dilutions, a qualitative test was first performed to find the optimal dilution ratio for a stronger, more homogeneous and thinner adhesive layer. Afterwards, quantitative experiments were performed on samples of silicones different base to binder and silicone to hexane ratios, to measure the compressive strength and modulus of elasticity of each.

- Qualitative:

Qualitative tests aimed to assess the effect of various Silicone to hexane ratios on PET binding to PMMA for various ratios namely 10:1, 10:2, 10:3, 2:1, 1:2, 3:10, 2:10, 1:10 and 1:100, according to the quality of the membrane assembly, the planarity of the surface, the homogeneity of the adhesive layer and the leakage to the duct area.

- Quantitative:

To assess the effect of the PDMS silicone to hexane ratio on the adhesive layer strength, for the chosen silicone base-to-binder ratios and silicone-to-hexane ratios, ratios (10:1 and 10:3) each with or without a PDMS silicone to hexane ratio of (1:2) are tested. Those four combinations are then quantitatively assessed using the mechanical loading and measurement Instron machine, where compressive strain was applied, and the compressive stress was measured on cylindrical shaped different specimens, three replicas to each condition. The specimen was a bone-shaped

having dimensions of (2.5 x 5 x 50 mm) according to the ASTM standards, and the tensile rate applied was equal to 1mm/min for 20 min, and the stress strain curve was assessed

iii. Results

After performing the qualitative testing on the PDMS silicones with different base to binder and ratios and silicone to hexane dilutions, the data was assessed visually for the homogeneity of the adhesive distribution and qualitatively comparing the binding force and the binding force distribution along the full surface interface in addition to the decrease in thickness for the same silicone hexane dilutions but different base to binder PDMS dilutions. And then the best PDMS dilution in hexane was chosen along with the standard 10:1 in (1:2) hexane and with 10:1 and 10:3 not diluted with hexane as controls. PDMS ratio and was then subjected to a quantitative compressive loading and measurement testing using the Instron machine, to obtain the stress-strain curve and get from it the modulus of elasticity of each of the materials tested.

- Qualitative:

For the qualitative test, the 10:1, 10:2 and 10:3 silicone to hexane dilutions resulted in no significant effect when compared to the non-diluted silicone combinations. While, the 3:10, 2: 10, 1:10 and 1:100 resulted in an inhomogeneous binding to all the areas of the two materials due to the low viscosity and the very low thickness of the resulting substance. The 1:2 silicone in hexane dilution was shown to be optimal when accounting to the strength of the resulting layer and the homogeneous and reduction of the thickness of the resulting adhesive layer. But it should be noted that that all the cases didn’t have good binding affinity to the PET and PMMA surfaces and thus further surface modifications is required.

- Quantitative:

The silicones were molded into four different 60mm in diameter petri-dishes and 12.3mm thick, four cases were studied which are the silicone base to binder ratios of 10:3 diluted in 1:2 silicone to hexane ratios, base to binder ratios of 10:1 diluted in 1:2 silicone to hexane ratios, base to binder ratios of 10:3 not diluted in hexane, and base to binder ratios of 10:1 not diluted in hexane. The resulting change in thickness was 1.6% for the PDMS 10:1 not diluted in hexane, 4.9% for the PDMS 10:3 not diluted in hexane, 65.8% for the PDMS 10:3 diluted in hexane at a ratio of 1:2, and 75.6% for the PDMS 10:1 diluted in hexane at a ratio of 1:2. Different samples are then taken from each of the molds and are subjected to compressive strain rates of 1mm/min for each 10mm overall thickness of the sample, thus accounting to the thicknesses of each sample, for example the 3mm thick sample was subjected to a strain rate of 0.3mm/min and the 12.1 mm thick sample was subjected to a stain rate of 1.21 mm/min. The samples are tested for different time points of 120s, 180s, 240s, 480s, and 300s, where 3 replicas were done for the 300s tensile experiments. Results had shown that silicone 10:3 had an overall decrease in thickness less than that of 10:1, but the compressive modulus of elasticity of 10:3 was higher than that of the 10:1 base binder ratios, and the case for the samples diluted in hexane. But an interesting observation was that for almost a 56.8% reduction in the thickness of the mold of the silicone 10:3 diluted in 1:2 in hexane, only 7% reduction in the modulus of elasticity was measured, compared with a 67% reduction in the modulus of elasticity for the 10:1 base binder ratios when diluted in hexane. Thus, 10:3 PDMS silicone base binder ratios was chosen and a dilution of 1:2 in hexane was shown to be the optimal dilution ratio.

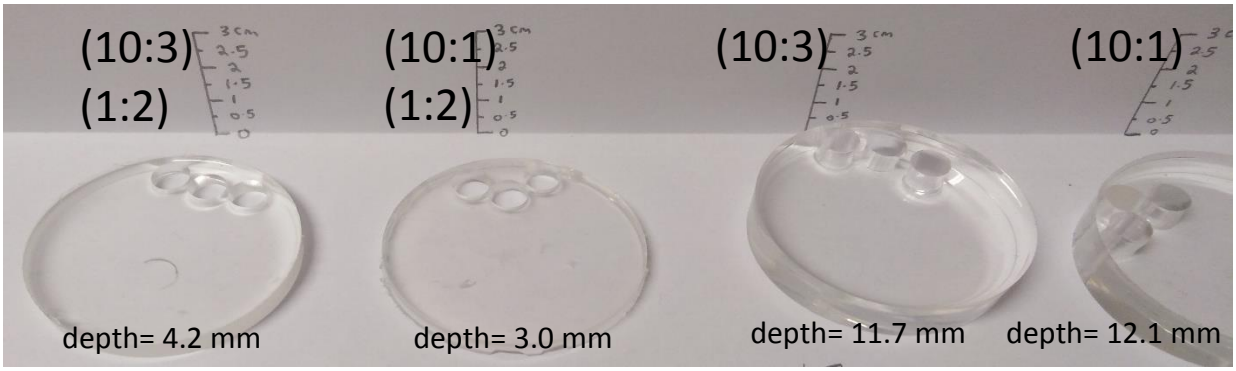


Figure 26 change in thickness of silicone combinations from 12.3 initially

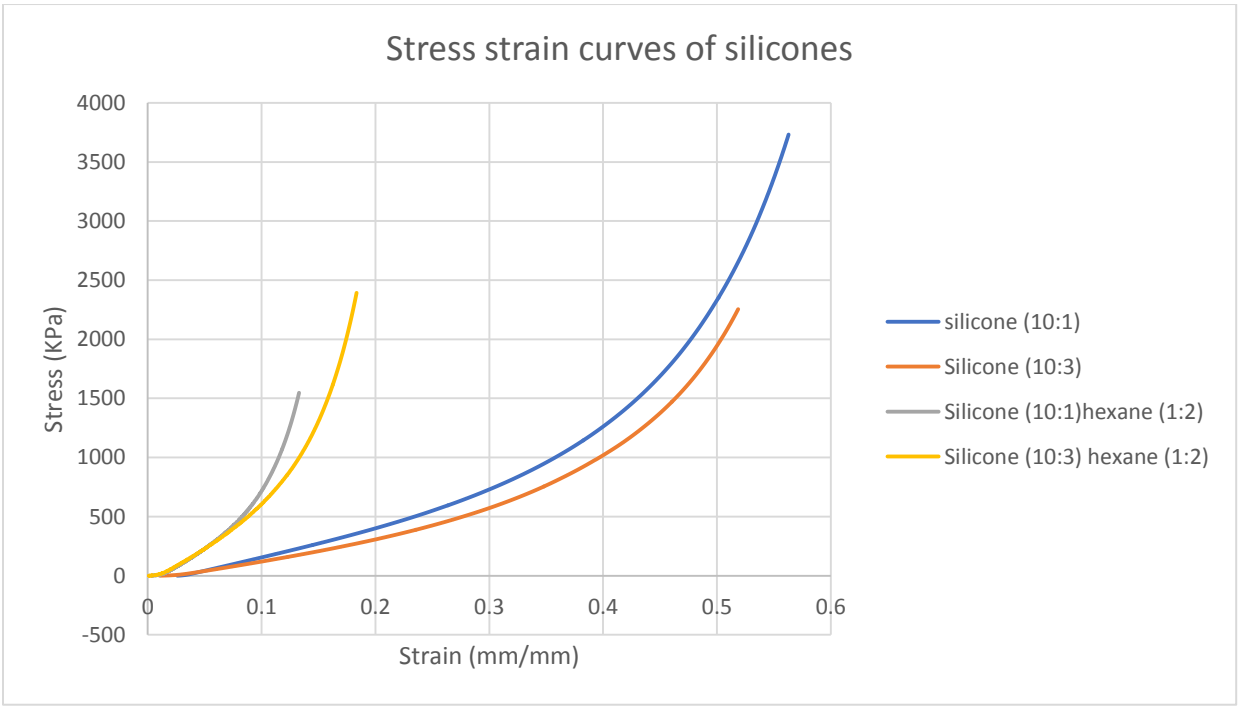


Figure 27 Compressive stress strain curves of different silicones and silicone hexanes ratios (compressive rates: 1mm/min for 300s (3 replicas).

Table 9 Compressive modulus of elasticity for an input strain rate of 1mm/ min and for 5 min (note: the rate depends on the initial thickness and was change relative to each thickness)

Treatment	Compressive modulus 300s (E) (Mpa)
Silicone (10:1)	19.8
Silicone (10:3)	12.8
Silicone hexane (10:1) (1:2)	6.6
Silicone hexane (10:3) (1:2)	11.9

c. Thermal fusion bonding at Tg (heat treatment: optimal temperature and time of treatment and cooling method)

Heat treatment of the materials (PET and PMMA) and curing of the PDMS at the glass transition temperature of the materials was used to reduce the errors of the machined parts, ensure a coplanar distribution and alignment of the PET membrane on top of the PMMA chassis, and at the same time to evaporate the hexane and cure the silicone. Since many parameters are included, then the optimization method was a bit complex since it is dependent on many different factors. But to be more flexible with the treatments that can fit for each of the indicated parameters, three heat treatment parameters were taken into consideration, which are the heating temperature and time and the cooling method.

i. Specifications

The heat treatment parameters and cooling method should be used to first reduce the errors of machining on the PMMA chassis to ensure the creation of flat planar surfaces, then it should be used at the different assembling stages, namely the PET membrane assembling to the PMMA chassis, and the two sub-assemblies assembling process binding the two membranes together. Thus, the chosen process and parameters should also result in the PET to PMMA and PET to

PET coplanar surface interface, the complete evaporation of hexane and curing of silicones, in addition that this temperature should be higher than the glass transition temperature of PET which is 80C to enhance its deformation into the cut chassis channels but should not exceed the melting temperature of PMMA which is 150C.

ii. Methodology

To find the effect of the different heating temperature and time combinations, we placed the assemblies in the furnace after using the optimal base to binder ratios and silicone to hexane ratios found before. The 37 different assemblies were placed in between two rigid flat plates (aluminum or glass) in the furnace, at temperatures of 80, 100, 120, 140 and 150C each for time periods of 10 min, 30 min, 1hr, 3hrs, 6hrs, 12hrs and 24hrs. And then they were assessed quantitatively for the change in thickness using an electronic caliper (resolution: 10um +/- 10um), and qualitatively for the distribution of the adhesive in between the layers and the homogeneous thickness of the adhesion and the binding affinity between the sub-assemblies.

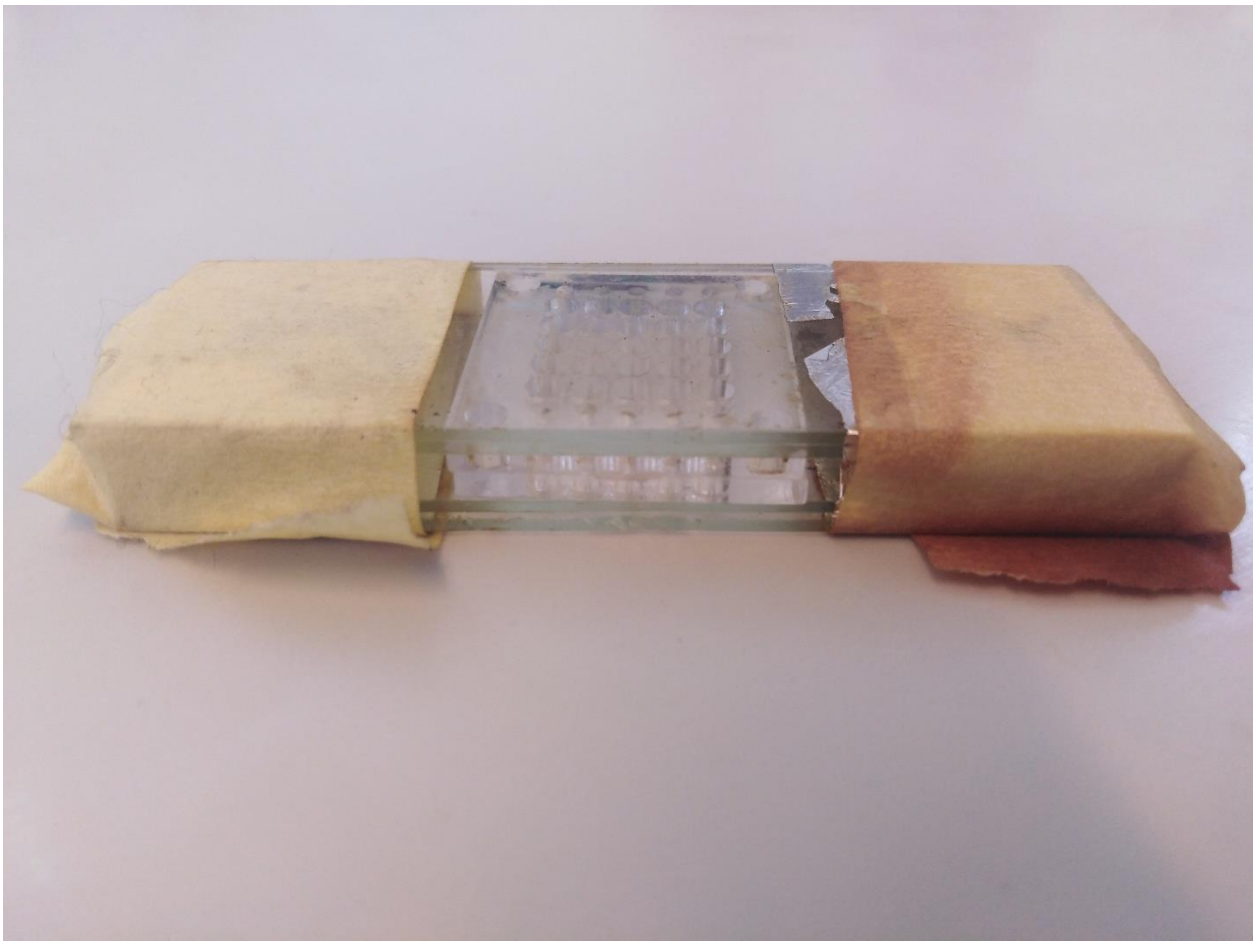


Figure 28 LOC placed in between two rigid flat glass plates

iii. Results

Results of the heat treatment experiments had shown that for the temperatures of 80C and 100C, the layers poorly adhered to each other even after extensive time periods of 24hrs, while temperatures of 140C and 150C resulted in significant reduction in the total thickness of the assembly up to 100um+/-10um for the PET and PMMA (4.8%) taking into account the change in the thickness of the PDMS adhesive layer (even for short heating time periods). On the other hand, a temperature of 120C resulted in both strongly adhered layers and negligible deformations. For the 120C heat treatment temperature, for heating times less than 12hrs, traces

of hexane still exist, and weak binding forces are resulted, while for extended periods of times larger than 12hrs, significant deformations occurred in the reduction of the PMMA components thickness.

d. Lamination, loading or pressurized bonding (preloading: weight and time)

Preloading is a vital part of the assembling process, where each part is to be loaded with weight while heat treating it. Pre-loading should be done at all assembling stages where the PMMA parts, the PMMA-membrane part & the full assembled LOC, are to be placed in between 2 smooth flat plates, & loaded with weights (fig.18 a). Various experiments are done Lamination and preloading the assembled parts to be cured is a key, since it is important to ensure that the surfaces are pressed against each other and the adhesive is completely and homogeneously distributed along the surfaces interface. But knowing the optimal lamination loads is very important to ensure a completely connected surfaces but with no effect resulting in material deformation or mechanical failure.

i. Specifications

Lamination should be done with the heat treatment at all the assembling stages, and the LOC should result in perfectly planar surfaces, but at the same time the resulting reduction in the LOC thickness should be negligible.

ii. Methodology

To assess the lamination effect and find the optimal lamination load, we placed weights on LOC while heating at 120C for 12 hrs. We then loaded the assemblies with 0.2, 0.5, 0.8, 1, 2, 3, 4, 5, 6, 8 & 10 Kg. Then we measured the resulting deformations with electronic caliper and we

visualized the membrane deformation under microscope. The heat treatment effect on the material properties and the effect of the cooling process on the pore size and the material molecular structure was then assessed using SEM imaging on the porous membrane after cooling it by quenching compared by slow cooling.

iii. Results

Results of the lamination testing showed that for small loads <3 Kg (47 KPa) for all 3 stages stated above, the LOC still showed non-planar PMMA surfaces, membrane-PMMA surface, & the 2 membranes adherent layer (fig. 10 b). For the 4, 5 & 6 kg loads (63, 78.5 & 94 KPa) the LOC showed perfectly planar surfaces with negligible reduction of LOC thickness (fig. 10 c). The 8&10Kg loads results in huge reduction in LOC thickness, up to 100um (2.5%) measured with an electronic caliper. Results of the SEM microscopy showed that when quenching (fast cooling of the material) in iced water, the material kept almost amorphous resulting in very low density of crystals on the surface. In contrary, for the slow cooled PET membrane (cooled in a period of 3hrs with the aid of an insulator), the membrane resulted in a very high density of crystalline structures on its surface. Results of the SEM microscopy on the change in the pore size for different cooling treatments showed that quenching resulted in very minor changes in pore sizes, while slow cooling resulted in a very high fluctuation in the pore size (+/- 1um) as shown in the figures below.

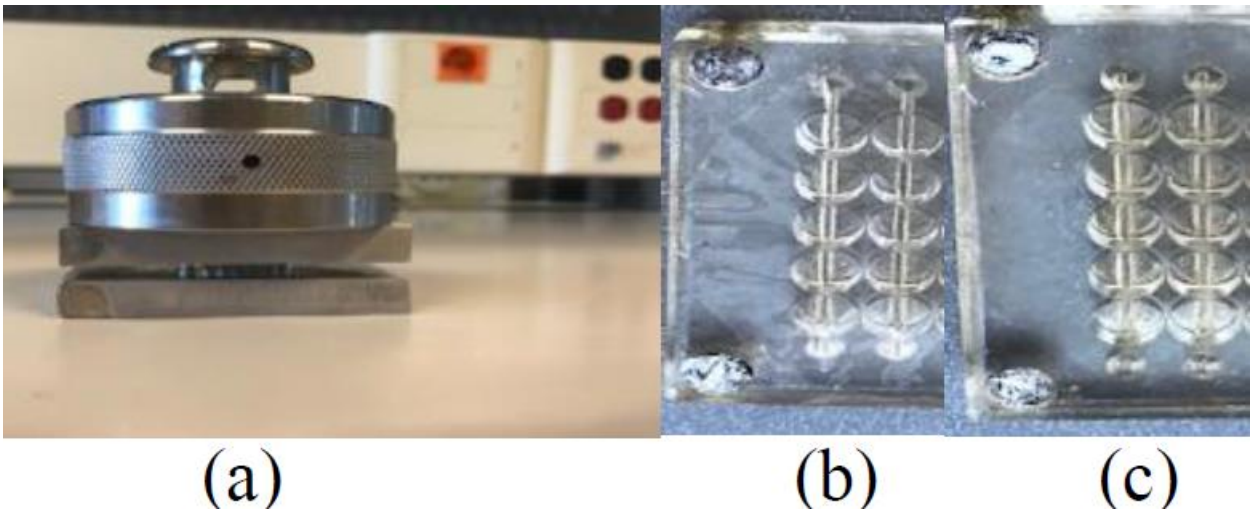
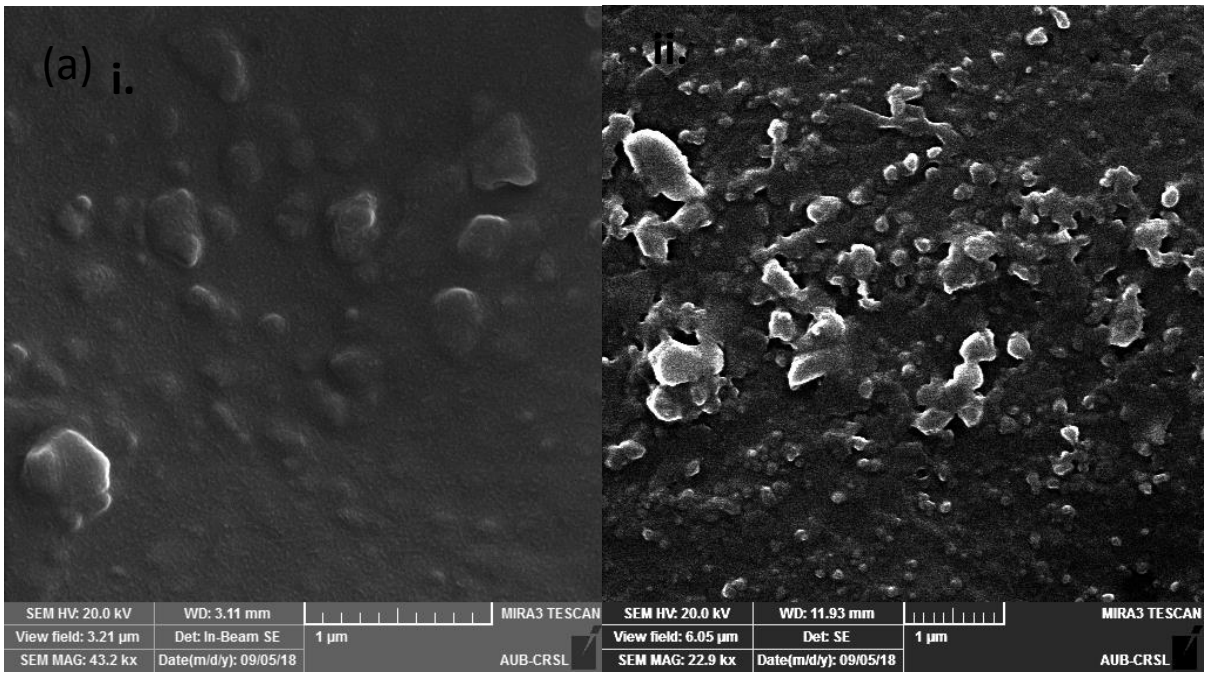
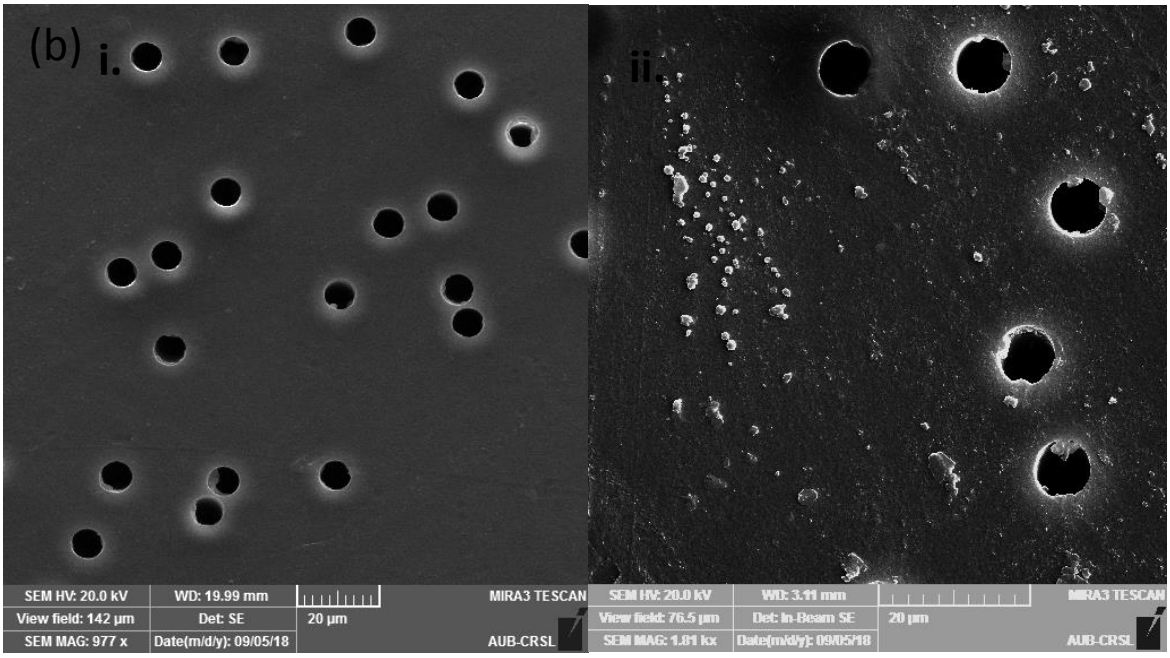
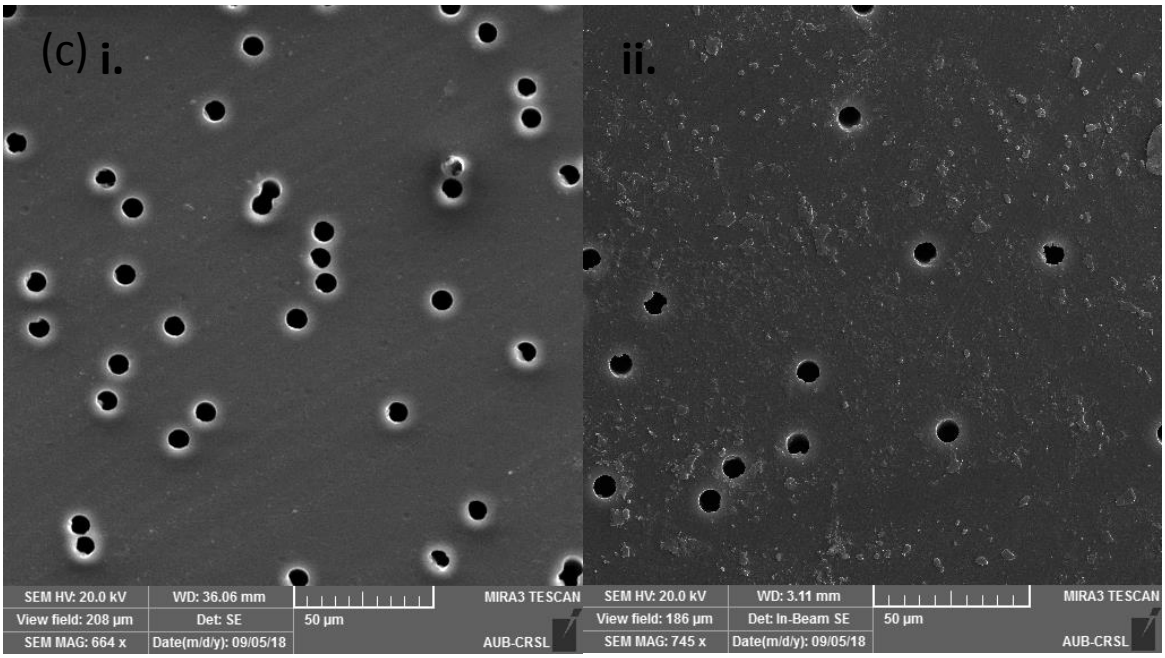


Figure 29 Preloading of LOC (a) 0.5 kg loading of LOC (b) LOC after 0.5 kg loading at 120°C showing deformed membrane (c) LOC after 5 kg loading at 120°C showing coplanar membranes




(b) i.
ii.
SEM HV: 20.0 kV
WD: 19.99 mm
MIRA3 TESCAN
View field: 142 µm
Det: SE
20 µm
SEM MAG: 977 x
Date(m/d/y): 09/05/18
AUB-CRSL
SEM HV: 20.0 kV
WD: 3.11 mm
MIRA3 TESCAN
View field: 76.5 µm
Det: In-Beam SE
20 µm
SEM MAG: 1.81 kx
Date(m/d/y): 09/05/18
AUB-CRSL


(c) i.
ii.
SEM HV: 20.0 kV
WD: 36.06 mm
MIRA3 TESCAN
View field: 208 µm
Det: SE
50 µm
SEM MAG: 664 x
Date(m/d/y): 09/05/18
AUB-CRSL
SEM HV: 20.0 kV
WD: 3.11 mm
MIRA3 TESCAN
View field: 186 µm
Det: In-Beam SE
50 µm
SEM MAG: 745 x
Date(m/d/y): 09/05/18
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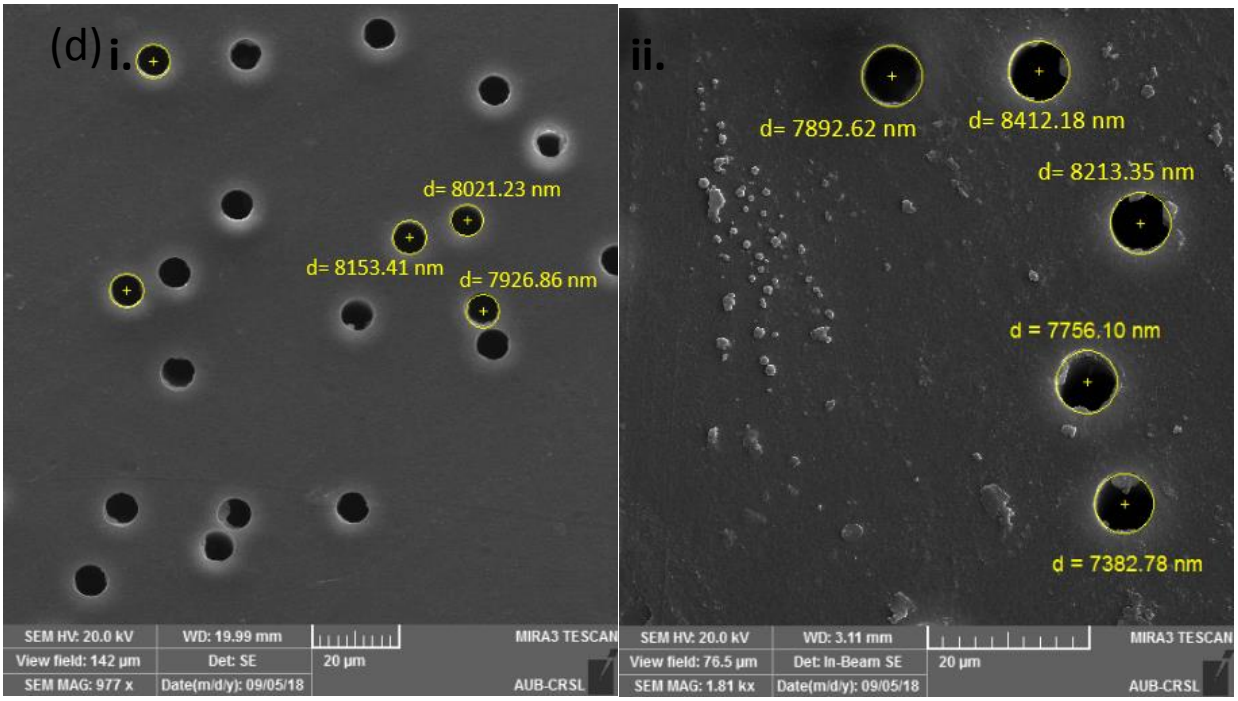

Figure 30 SEM microscopy imaging for (a) 1um scale microscopic structure i. quenched membrane was almost amorphous with minor crystallization ii. Slow cooled membrane was almost fully crystalline with large crystal sizes. (b) 20umand (c) 50 um scale microscopic structure both showed respectively that i. quenched membrane was almost amorphous with few to no crystal formation ii. Slow cooled membrane was of highly concentrated crystalline structures. (d) 20um scale microscopic structure and pore sizing showed that i. quenched membrane resulted in minor deformation in the pore size while ii. Slow cooled membrane resulted in a large range of pore size fluctuation (+/-1um)

e. Surface modification (O2 plasma treatment: time)

Initial experiments using silicone as an adhering layer between two PET surfaces or between PET & PMMA surfaces, showed that the layers could be easily disassembled, especially after dipping it in water or ethanol. The reason is the non-adherence of the silicone layers to one or both PET & PMMA surfaces. Different treatments were done on the surfaces to aid the adherence of the silicone on it. One significant treatment was the acetone surface etching, which results in the adherence of the silicone on the surfaces, but results in the blurriness of the PMMA surface. Thus, plasma surface etching (fig.19) was done to solve the problem without effecting transparency.

i. Specifications

For the plasma treatment, it should be done in all the assembling stages similar to the heat treatment and the lamination preloading, in addition, the LOC should show a perfect distribution of the adhesive and thus in an enhanced adherence of the silicone to the PMMA and PET surfaces.

ii. Methodology

4 surfaces are placed in the plasma machine at 2, 4, 8, & 16 min respectively (n=3). And the results are assessed qualitatively according to the enhancement in the binding of the silicones to the PET and PMMA surfaces. This could be clearly observed by the homogeneous distribution of the adhesives throughout the treated surfaces, and the increase in the binding forces.

iii. Results

Results shows that 2 & 4 min treatment, results in a minimal effect on the silicone adherence to surface, while that of 8 min showed much better effect, where silicone adhered smoothly & easily directly after treatment. The 16 min treatment showed better adhesion, but results in significant amounts of impurities from the air dust.

f. Controlling silicone spreading along wettable areas (into the duct)

After treating for leakage prevention, we ensured the precise LOC assembly without impurities, adhesive nonhomogeneous distribution, chip non-planarity & membrane deformations. But another problem popped up, which is the narrowing or even clogging of some of the ducts, due to either the silicone being pushed to the direction of the duct when loaded and treated, or the membranes forming the ducts deforms at a specific temperature, causing fluctuations in the duct diameter. One major problem occurring is that when the binding of the silicone was enhanced

through the surfaces treatments specifically the O2 plasma treatment, the risk of the silicone expansion to the ducts and thus blocking the ducts increased. After many trials that includes reducing the temperatures & silicone amount used, it still results in leakage, improper connectivity, and inhomogeneity. Thus, we proposed a new solution that goes by passing nylon wires having an outer diameter equal to the inner diameter of the ducts (500um) while assembling the LOC).

The aim is to ensure that there will be no pathway for the silicone to pass to the ducts, and the duct membranes to shrink & deform. Thus, the inserted wire should Block the pathway into those areas and coating those areas with cells adherent substances above the silicone. And thus, to ensure that there will be no pathway for the silicone to pass to the ducts, and the duct membranes to shrink & deform. The nylon wires are then to be removed prior to the LOC usage, as they can be easily pulled out of the ducts using tweezers.

Figure 31 Passing wires in ducts (a) inserting the wires in position (b) assembling LOC with wires (c) wires in LOC after assembling and treatment (d) removing the wires from ducts prior to LOC usage

4. *Leakage Testing*

After doing the above optimization and testing processes, we performed a leakage testing on the LOC to make sure that no leakage occurs. We passed colored water, in the ducts, using a syringe

pump (flow rate=30um/min), we then checked for leakage. Knowing that our design process is based on iteration, then leakage testing is the key mechanistic test needed

a. Inter-Ductal and Intra-Ductal Leakage

All the manufacturing and assembling process after optimization of each process or step, should be tested for mechanistic functionalization to assess the detailed LOC design. One very important risk limitation is the risk of inter-ductal leakage of the leakage in-between the ducts due to the inhomogeneous assembling or adhering, or the intra-ductal leakage or the leakage across the duct due to duct breakage. Leakage testing was done to assess the leakage in the system and its causes. Leakage testing is part of the iteration process and thus should be done after each assembling stage.

i. Specifications

The system should be of optimal and have a homogeneous adhering layer, and to assess the planarity and the homogeneity of the adhesion layer, we should perform leakage testing and test if inter-ductal leakage happens. Thus, for the leakage test, the leakage should not occur in between the ducts, and to test if the membrane is not broken, the leakage should not happen across the duct to the wells.

ii. Methodology

The method of the leakage testing is by pipetting the 15 uL of colored water (Coomassie blue) into each of the ducts inlet and wait for 3hrs to see if inter or intra ductal leakage happens.

iii. Results

After the final optimization on the assembling process, when passing colored water into the ducts, 5 straight and smooth lines appears showing that no leakage happened.

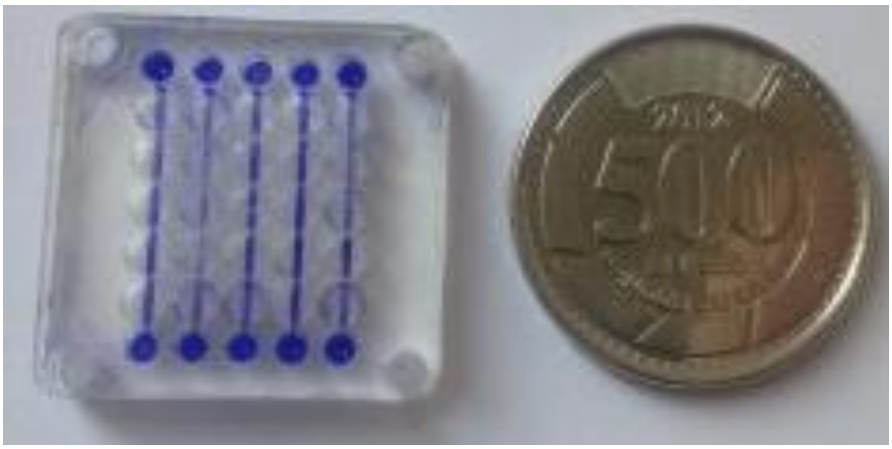

Figure 32 Inter-ductal and intra-ductal leakage testing (no leaking)

b. Tubing and Connectors Leakage

i. Specifications

The system should be of optimal in terms of connections and connectivity, and thus to assess the connections functionality, leakage testing should be done while the LOC was assembled to the pump and tubing. The System should not leak from anywhere along the connections from the pump reaching the LOC inlet.

ii. Methodology

The LOC was connected to the holder and tubings and the tubings were connected to the pump, and from there it was connected to the syringe tip held on the syringe pump. Then leakage was assessed visually to check if any leakage happens at the connectors' stage, and thus may risk contaminations for future applications.

iii. Results

After the final optimization on the assembling process and the connections, when passing colored water through the micro-pump at a rate of 100uL/min, no leakage drops happened at the level of the connectors.

B. **Biological Testing**

After assessing the LOC mechanistically, it should now be assessed biologically for cell viability and attachment. The key is to first optimize the cell culturing procedure on the LOC through performing cell culturing processes on the surface materials of the chip with the materials that should be used in the culturing process and assess their effect, and then assess the binding enhancing parameters. Then after that a procedure would be done for the cell culturing process in the LOC.

1. *Cell Attachment and Viability on Various Surfaces and Coatings*

It is important to assess cells functionality along with different substances that are to be used in the culturing process, in order to better understand the limitations of each feature to be used while designing a biological experimentation procedure for our LOC. To do so, we should assess the effect of the PET membrane soaked with ethanol, since it is the substance we use to sterilize the LOC, and then we should assess the effect of treating the surface with cell media to enhance the binding and viability of the cells.

a. Specifications

Maximum adhesion should occur while assessing the effects of soaking the membrane with ethanol to sterilize it on the cell viability and adhesion on the material surface, since we don’t

know how the cells would interact with the material surface and how the ethanol would interfere with the materials found and lead to the release of substances toxic to cells. In addition, we should assess the effect of treating the surfaces with media to enhance the cell viability and binding to the surfaces.

b. Methodology

Cell should be cultured on top of membranes with various treatments, one soaked only with ethanol, another one as a control and not soaked with ethanol, and one soaked with ethanol but treated overnight with cell media, and another also as a control where the membrane was not soaked with ethanol but was treated overnight with media.

c. Results

Results on the PET membrane soaked with ethanol Showed a decrease in cell growth in compared with the other one not soaked with ethanol. While the effect of the treatment with media soaking overnight had no effect on membranes not soaked with ethanol, it enhanced the cell growth on membranes previously soaked with ethanol.

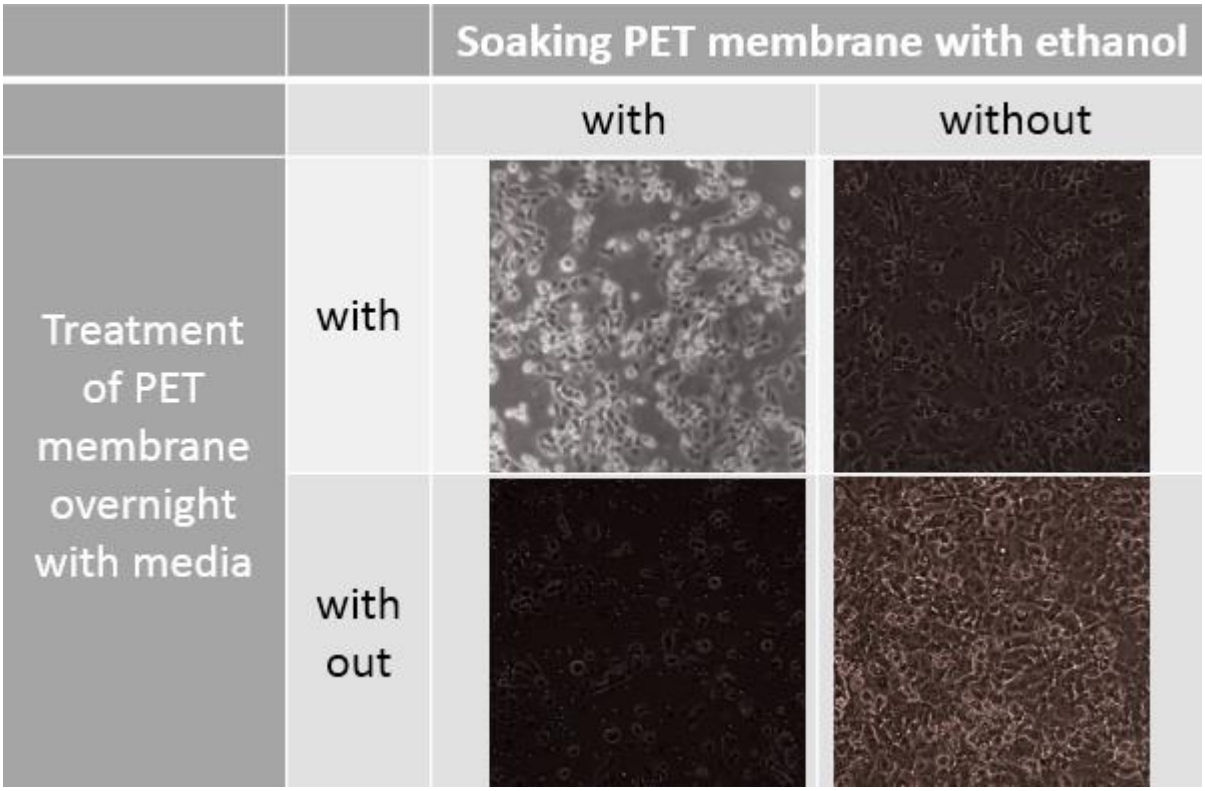

Figure 33 Cell viability and adhesion on surfaces sterilized with PDMS and that treated with cell media.

2. *Cell Attachment Quantification & Optimization after Various Washing Times*

In order to develop a procedure for the cell flow inside the LOC ducts that are feasible for the cells to attach on the walls of the ducts, we should first assess the time needed by the cells to attach on the surfaces without flow.

a. Specifications

We need to find the optimal time needed for the cells to stay on the surface and adhere to it. Thus in this experiment, the optimal washing time of cells is the time at which minimal cell loss occur while washing.

b. Methodology

In this experiment, 500,000 MDA-MB-231 cells were seeded on top of a 6 well plate surface and then washed after a specific time period of 1.5, 3 or 6 hours and one well was left as a control. An n=3 was done on this experiment and the cell count was assessed using trypan blue cell counting assay.

c. Results

Results had shown that a 6-hour washing time on the cells resulted in number of living cells similar to the control (around 85% still attached) in contrary to the washing time of 1.5 hours where almost only 20% of the cells still attached and the 3 hours washing time where around 60% only attached.

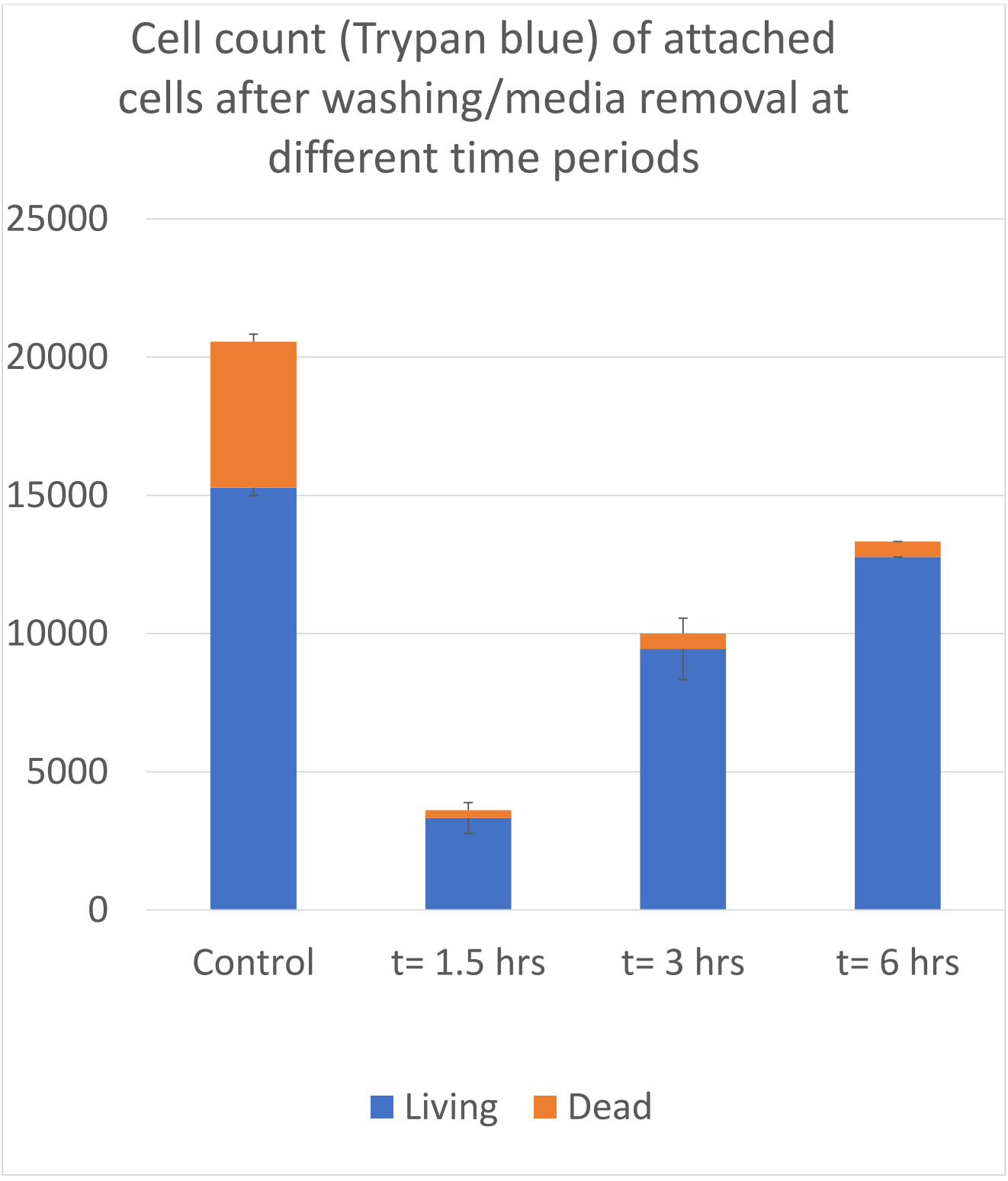

Figure 34 Trypan blue cell counting assay on the effect of the washing times on the cells attachment to the surface.

3. *Cell Viability and Attachment on Duct Wall of LOC*

The final experiment being done is the cell viability and attachment on the inner walls of the LOC duct. In this experiment biological assessment of the functionality of the LOC was done and accordingly further modifications of the design should be done.

a. Specifications

Cells are passed in the duct at a should bind to the surfaces of the duct to later grow and form the epithelial tissue of the duct.

b. Methodology

Cells are passed in the duct at a concentration of 1000000 cells/ml and at a flow rate of 5uL/min and kept for 24 hours. Then the LOC ducts were visualized under the microscope for cell adhesion.

c. Results

Results had led to an unknown or unconfirmed assessment, due to the complexity of the biological process and the many different parameters that may play a role in this process such as leakage, auto-fluorescence, debris or other unknown parameters, and thus further investigation and experimentation should be done, by performing more controlled experiments.

CHAPTER VI.
CONCLUSION & FUTURE PERSPECTIVES

In this thesis, we had developed an LOC that is first to mimic the full 3D structure of the duct, by using an iterative design method, by which modifications are done at each step and new materials and methods are proposed diverse from the current LOC design methods. Experiments are still ongoing to find the optimal LOC assembling and solve the cell binding problem to the inner walls of the ducts. For the material iteration, PEEK or PET should be used for the chassis instead of PMMA, since PEEK has superior properties that fits well in the set material specifications, and PET also have better properties comparted to PMMA and increase our modification options since it the same material as the membrane. Silanes should be used to optimize surfaces for adhesion to PDMS, thus reducing many of the current problems. In addition to different PET-specific high strength & low viscosity thermally cured adhesives that may be used instead of PDMS if current problems persist. For the manufacturing process, we aim to create a cheaper, faster fabrication & assembling process of the LOC, (other than CNC & hand assembling) to be applicable for mass production. In addition, work is currently being done and is to be completed, on the CFD analysis on the porous duct to find the relation between the mass flow rate through the duct and the mass flow rate across the duct for culture fluids, and find the pressure distribution along the duct, to get the optimal parameters for the cells to live and attach. As for the biological testing, the LOC is to be tested for single layered mammary ductal formation, and then for co-culturing both the normal and tumor cells, to mimic the environment in ductal carcinomas, and asses the effect of various drugs on tumor growth & invasion. Future

work may be done to design and fabricate a membrane-less ductal system LOC that may even mimic more the in-vivo micro-environment.

As a conclusion, the proposed LOC can be adapted on various ductal systems such as breast, endothelium, lung, kidneys, and pancreas tissues, among others, as it can be easily manipulated to assemble different cells, ECMs and duct sizes, with varying media components and flow rates. In addition, this LOC can also be developed by pharmaceutical companies for high-throughput testing of drugs' efficacy, delivery, and targeting in ductal systems.

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