

Fabrication of microfluidic chip using 3D printing for ocular cell studies

by

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A thesis

presented to the University of Waterloo

in fulfillment of the

thesis requirement for the degree of

Master of Science

in

Vision Science

Waterloo, Ontario, Canada, 2023

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Author's Declaration

I hereby declare that I am the sole author of this thesis. This is a true copy of the thesis, including any required final revisions, as accepted by my examiners.

I understand that my thesis may be made electronically available to the public.

Abstract

Purpose:

This thesis aimed to create a polydimethylsiloxane (PDMS) microfluidic chip utilizing low-cost commercial 3D printers and to integrate human corneal epithelial cells (HCEC) into the fabricated chip.

Methods:

The purpose of the first experimental chapter (Chapter 3) of this thesis was to develop PDMS devices from 3D printed moulds of two commercially available 3D printers: the Formlabs Form 3B+, which employs Stereolithography (SLA) technology, and the AnyCubic D2 DLP, which utilizes Digital Light Processing (DLP) technology. The fabrication of PDMS from 3D printed moulds can be achieved by introducing three simple post-processing steps: heating, sanding, and nail polish coating. A biomaterial coating was applied to the surfaces of the PDMS devices to render their surfaces hydrophilic.

The second experimental chapter (Chapter 4) centred on incorporating HCEC into the PDMS device fabricated using the method described in Chapter 3.

Results:

Both 3D printers could generate optically clear PDMS devices with smaller channel dimensions of 100 μm , with faster print times and higher accuracy for the DLP 3D printer. Heating the 3D printed moulds produced fully cured PDMS chips with

perfect channel edges. The sanding and nail polish coating of the mould produced optically transparent and smooth PDMS devices. Coating the PDMS surface with polydopamine (PDA) improved its surface wettability from 110° to 75°.

On the PDA and collagen-coated device, HCEC exhibited significantly better growth than on the untreated PDMS device. HCEC cultured on these PDA/collagen-coated PDMS devices demonstrated 80% cell viability compared to conventional tissue culture plates.

Conclusion:

This study demonstrated that SLA and DLP printers can be used to produce PDMS microfluidic chips quickly and affordably. Notably, the DLP printer offered better accuracy and faster printing time than the SLA printer. The fabricated PDMS chips were transparent and capable of incorporating HCEC.

Acknowledgments

I am humbled and profoundly grateful for the invaluable support and guidance from my co-supervisors, Dr. Lyndon Jones and Dr. Chau-Minh Phan. In the realm of research, I was like a newborn, eager to learn and explore. They firmly held my hand, teaching me to kneel, crawl, and eventually take confident steps. Their patience during learning and constant motivation during difficult moments have been indispensable.

I would like to extend my deepest gratitude to Dr. Carolyn Ren for generously sharing her expertise in microfluidics. Also, I sincerely thank Dr. Vivian Choh and Dr. William Ngo for graciously imparting their knowledge in cells, with special mention of their insightful feedback, which was invaluable.

I am indebted to Dr. Brandon Ho for his direction and support throughout the project and for sharing his cell expertise. A special mention to Dr. Ning Qin for generously taking his time and effort to transfer his skills in microfluidics fabrication. In addition, I would like to thank Dr. David Wulf for sharing his mastery in 3D printing.

Finally, I would like to express my heartfelt appreciation to my parents and friends for their moral support and constant motivation.

Dedication

This thesis is dedicated to my parents, mentors, and friends whose unwavering support and guidance were instrumental in shaping my progression.

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List of Abbreviations

Col	Collagen
2D	Two-dimension
3D	Three-dimension
CAD	Computer-Aided Design
DLP	Digital Light Processing
DMEM	Dulbecco's Modified Eagle Medium
FDA	Food and Drug Administration
FDM	Fused Deposition Modelling
FIH	First-in-human
GelMA	Gelatin Methacrylate
HPLC	High-Performance Liquid Chromatography
HTS	High Throughput Screening
MSLA	Masked Stereolithography Apparatus
NDA	New Drug Application
PBS	Phosphate Buffer Saline
PDMS	Polydimethylsiloxane
PMMA	Polymethylmethacrylate
RPMI	Roswell Park Memorial Institute
Rq	Root mean square roughness

Si	Silicon
.stl	Standard Tessellation Language
SLA	Stereolithography
VA	Visual Acuity
WCA	Water Contact Angle
WHO	World Health Organization

1 Introduction

1.1 Challenges in the development of effective treatments for ocular diseases

1.1.1 Impact of vision loss and the need for effective treatment

“The eyes are the windows to your soul,” said William Shakespeare, one of the greatest writers of the 16th century.¹ The eye is one of the most crucial organs of the human body that allows us to perceive the world around us.² The light enters through the cornea, the transparent portion at the front of the eye, and is focused by the crystalline lens onto the retinal photoreceptors. These photoreceptor cells transform light signals into electrical impulses that reach the brain via the optic nerve, which are then interpreted as three-dimensional (3D) images.³ The visual perception process requires anatomical integrity and physiological interconnection between the brain, eyes, and their related structures. The disruption of any of these components in the visual pathway can lead to vision loss and, potentially, even blindness.³

The eye is also closely connected to the entire human body. Systemic diseases, including hypertension, diabetes, and autoimmune diseases, are linked to the onset of eye-related diseases.² The complete loss of vision or vision deterioration impacts the quality of life in areas of independence, mobility, mental cognition, education, employment, and social life.⁴ Furthermore, the economic burden associated with vision loss is significant.⁵ According to the 2013 study commissioned by Prevent Blindness, the total tangible and hidden expenses associated with vision loss in the United States were estimated to be \$138 billion in 2013 and estimated to expand up to \$717 billion by the year 2050.⁴⁻⁶

The World Health Organization (WHO) characterized the occurrence of low vision and complete absence of vision according to visual acuity (VA) (Table 1.1).^{7, 8}

Visual acuity is the measure of the ability to differentiate the finest features of a high contrast target at a standardized distance and lighting conditions.⁹ Snellen is the most commonly used VA chart, denoted as the fraction of test distance to the tiniest line correctly read.³ According to the Canadian Council of the Blind, as of 2021, 1.2 million Canadians (3.2% of the total population) have significant vision loss or blindness.¹⁰ By 2050, it is projected that globally, 474 million will be visually impaired, and 61 million will be affected by complete vision loss.¹¹ 40% of irreversible visual impairments were caused by cataracts and visual pathway diseases.¹² Age-related macular degeneration, glaucoma, and diabetic retinopathy were the subsequent leading causes of blindness.⁷ Research with innovative approaches and utilization of new technologies such as gene therapy and stem cell therapy are crucial in advancing global eye health, providing effective treatment, and mitigating irreversible vision disorders.³

Table 1.1 – WHO classification for visual impairment based on visual acuity (VA)

Category	Types of vision	Visual acuity (VA)	
		Snellen (in meters)	Snellen (in feet)
Normal vision	0 - Normal vision	6/7.5 or better	20/25 or better
	0 - Near normal vision	6/9 - 6/18	20/30 - 20/60
Low vision	1 - Moderate low vision	6/21 - 6/48	20/70 - 20/160
	2 - Severe low vision	6/60 - 3/60	20/200 - 20/400
	3 - Profound vision loss	6/150 - 6/300	20/500 - 20/1000
Blindness	4 - Near total blindness	< 6/300	< 20/1000
	5 - Total blindness	No light perception	No light perception ^{3, 7, 13}

1.1.2 Stages of ocular drug development process: From preclinical development to clinical trials and regulatory approval

Developing new ocular drugs and medical devices is a demanding process that takes 12 to 15 years on average and exceeds \$1 billion in expenditure per drug.¹⁴⁻¹⁶ For a new ocular drug to enter the market, it must pass through various stages, starting from preclinical development to clinical trial phases, followed by regulatory clearance through agencies such as Health Canada and the Food and Drug Administration (FDA) and, finally, successful aftermarket surveillance (Figure 1.1).¹⁷

1.1.2.1 Drug discovery and preclinical development

The drug discovery process aims to identify a highly safe and effective drug for treating a targeted disease. Additionally, it must have the highest potential for market launch to ensure appropriate financial return.¹⁶ It is estimated that only 1 in every 5000 drugs evaluated in the drug discovery phase enters the market.¹⁸ The drug discovery process involves numerous phases, including target identification and validation, high throughput screening (HTS), hit identification, assay development, lead generation, and optimization, followed by preclinical testing (Table 1.2).^{16, 19-21}

The target identification process begins with a detailed comprehension of biological and physiological mechanisms, pathways involved in the disease, as well as potential hereditary and environmental risk factors that might lead to the disease condition.^{18, 22} The potential drug target should uphold clinical and commercial standards, be safe, and effective.^{16, 23} Using advanced methodologies and scientific technology, the drug discovery process entails the identification of potential therapeutic targets. Once the ideal therapeutic target has been determined, it can be validated to ensure the repeatability of its functional activity in treating the specific disease condition.^{23, 24} Subsequently, the potential lead candidate can be identified

and optimized to ensure that it is most likely to succeed in clinical trials. The selected drug candidate is characterized for its toxicity, structure, size, and improvement of the disease targeted.²³ This leads to the essential crucial next step of preclinical testing, where the safety and effectiveness of a drug are examined in animal models. It involves two main areas of study: pharmacology and toxicology. Pharmacology focuses on the pharmacokinetics (study of how the body interacts with the administered drug) and pharmacodynamics (understanding the biochemical and physiological effect of the drug on the body) parameters of the substance. In contrast, toxicology studies the toxicological effects of the drug *in-vivo*. The preclinical trials must be approved by regulatory authorities, ensuring they can be conducted safely and ethically, and only drugs that are safe and effective are finally approved.^{16, 18, 24}

1.1.2.2 Investigational New Drug (IND) application:

Once the desired drug candidate has been demonstrated to be safe and effective in preclinical studies, an application is submitted to the regulatory bodies for the initiation of human trials by the drug developers or industrial sponsors.^{23, 25} The FDA in the United States and Health Canada in Canada are the regulatory bodies that authorize the testing of preclinically validated therapeutics in human clinical trials.²⁶ The IND application requires information on pharmacology and toxicology study results in animal models, detailed information on drug manufacturing procedures, and information pertinent to clinical trial procedures and investigators involved.²⁵

Table 1.2 - Stages in drug discovery process from target identification to preclinical drug development.

Stages	Description
Target identification	Identifying potential therapeutic targets, typically through tools such as data mining, bioinformatics, functional screening, and phenotypic analysis.
Target validation	Validate the selected potential target for functional activity towards the specific disease.
High throughput screening (HTS)	Screening several thousands of compounds (chemical libraries) against the target (specific biological molecule or pathway) to identify the potential hits with activity.
Hit identification	Hits identified through HTS can be validated for their potency, selectivity, and pharmacological properties. It involves secondary screening, medicinal chemistry optimization, and structure-activity relationship studies.
Assay development	Developing specific assays to evaluate the efficacy and activity of selected compounds. Cell-based assays measure compound activity within cells, while biochemical assays measure the binding affinity between the compound and target protein.
Lead generation	Compounds with the highest activity, selectivity, and safety among hits are identified as "leads."

Lead optimization	The objective of this step is to preserve the beneficial properties of the lead compound while resolving its deficiencies. The lead compound undergoes an iterative change to improve its efficacy, selectivity, adsorption, distribution, metabolism, excretion, and toxicity (ADMET).
Preclinical studies	Evaluating optimized lead compound for its safety and efficacy in cell cultures and animal models. ^{16, 24, 27}

1.1.2.3 Clinical trials

Clinical trials are scientific investigations undertaken on humans. Their primary objective is to assess the safety and effectiveness of new medical therapies. These trials aid scientists in determining whether novel treatments/therapies are successful and safe compared to existing solutions. They can be classified into four phases, from I to IV.^{28, 29} The preclinical studies are followed by **First-in-Human (FIH) trials** and serve as a prelude for the beginning of human clinical trials.²⁶ In FIH trials, a tiny group of healthy volunteers or diseased subjects (10 to 15) are administered the drug in escalating dosages to determine the maximum tolerated dose, and any adverse effects are recorded.^{24, 26} The aim of a **phase I** clinical trial is to determine the drug's safety, dosage, pharmacodynamic and pharmacokinetic profile, and any potential side effects in a small group of healthy volunteers (20 to 80).^{23, 30} In terms of investigations involving intravitreal administration of an ocular drug, this involves phase 1a/2b, which evaluates the safety and effectiveness of an ocular drug, first in healthy volunteers (Phase 1a) and then in individuals with the targeted ocular condition (Phase 2b).³⁰ In **phase II** clinical trials, up to 300 individuals are evaluated for the efficacy and additional safety of the drug. Here, the medication or treatment is generally considered against a placebo with a follow-up period of 2 years, and a typical success rate of 33% is reported.^{23, 30} In **phase III** clinical trials, a large group (300 to 3000) of diseased individuals are tested to determine the drug's efficacy for 1 - 4 years. In this stage, the therapeutic medication or treatment will be compared with already established therapies, any potential side effects will be observed, and the overall balance between the benefits and risks of the treatment will be assessed.³¹

After the successful completion of phase III clinical trials, a **New Drug Application (NDA)** is submitted to the relevant regulatory agency by the drug

developers or industrial sponsors to approve the new drug or treatment and its release into the market. This application includes all pertinent information about the drug, including preclinical testing to phase III clinical trials, insights about the drug's chemistry and manufacturing details, suggested packaging and labelling methods, and patent information.²³ The review process varies, and it might take up to a maximum of 15 months. On approval, the drug enters **phase IV**, also termed post-market surveillance, which involves several thousand volunteers with the diseased condition. This phase aims to understand the drug's long-term safety and efficacy and discover any unidentified side effects in the previous clinical studies.^{24, 31} The outcome of this phase results in negotiation with the regulatory agencies and sponsors to update the package labelling and train the health care professionals on how to employ this medication to treat the disease.²⁴

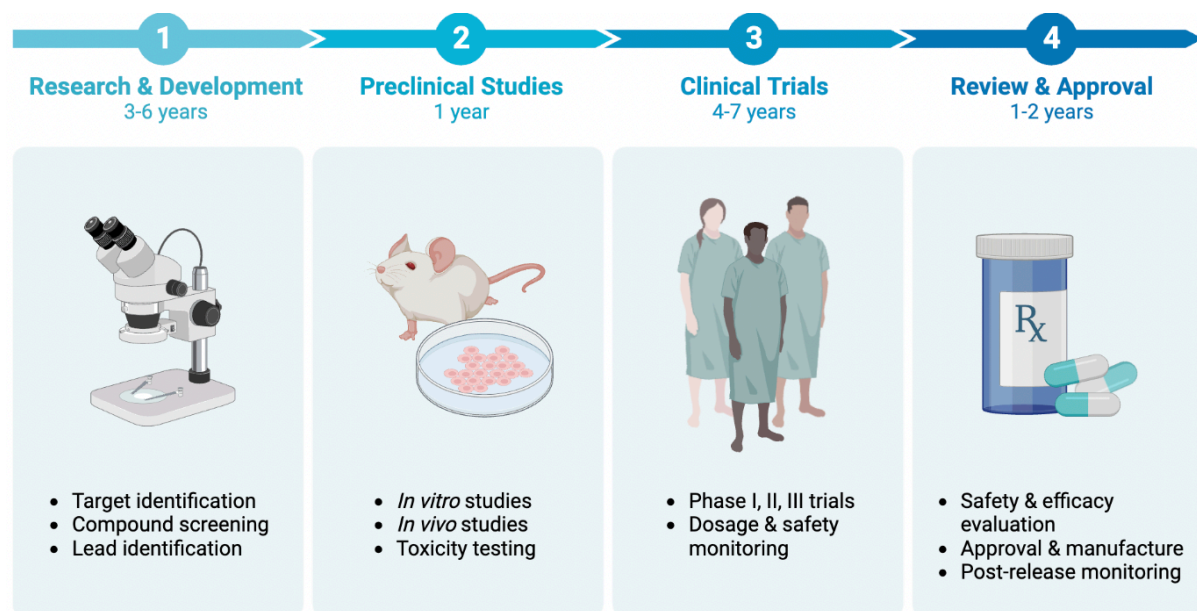


Figure 1.1: Overview of the drug discovery process.
(Created with Biorender.com)

1.1.3 Ineffective preclinical testing and clinical trials failure

Preclinical testing aims to identify the drug candidate with the highest possibility of becoming a commercially viable product while being remarkably safe and efficacious. The preclinical studies are a crucial step, bridging the drug discovery process and clinical trials.³² However, 90% of drug targets that succeed in preclinical testing fail to pass clinical trials. One main reason is the unforeseen toxicity (33%) and efficacy (43%) during the early stages of testing.^{33, 34} Approximately one-fifth (20%) of the entire expenditure on drug development is reserved for preclinical research, with clinical trials accounting for four-fifths (80%) of the expenses.³²

Traditionally, pharmacokinetic studies performed by blood analysis are inappropriate for ophthalmic drug research. The regulatory bodies encourage a range of bioequivalence investigations, such as pharmacokinetic studies in aqueous humour, clinical endpoint studies, microbial kill rate assay, and *in vitro* release studies.³⁵⁻³⁷ *In-vitro* studies are considered more reliable for evaluating ocular therapeutics in the early stages of testing. There have been attempts to develop physiologically pertinent computational models. For instance, the Ocular Compartmental Absorption and Transit (OCAT™) model could predict the bioavailability of ocular drugs and tear film break-up time.^{35, 38} Additionally, instruments such as the Franz diffusion cell are utilized for the *in-vitro* evaluation of ophthalmic ointments and suspensions.³⁹ This device consists of two segments separated via a membrane. The membrane acts as a barrier, allowing the diffusion of drug formulation from one end to the receptor fluid on the other side.^{35, 40} Though *in silico* modelling and *in-vitro* studies could expedite preclinical drug screening, they cannot predict drug behaviour in a complex physiological environment.⁴¹ Hence, experimental evidence from animal models is required.⁴¹

Since 1964, using animal models such as live rabbits and dogs for eye-related studies has been accepted by regulatory bodies.⁴² Furthermore, the *in-vivo* Draize test is claimed to be the gold standard method for determining acute ocular toxicity. In this method, 100 μ L of the investigational agent is applied to one of the rabbit's eyes and observed for ocular discomfort after 72 hours.⁴³ However, using animal models exhibit variations in anatomical and physiological parameters, blink interval, and tear flow dynamics when compared to the human eye.⁴² For instance, when compared with humans, the rabbits' epithelial layer (10 times) and Bowman's layer (6 times) are thicker (resulting in a higher pain threshold), and the rabbit has a very different tear composition and structure to humans.⁴⁴ Additionally, utilizing animal models raises severe ethical concerns. It must follow the 3R's guideline of European Union (EU) legislation, which is to 'replace' – explore other options, 'reduce' – minimize animals used and 'refine' – minimize animal suffering.³⁵

To reduce the use of live animals in experimental studies, excised bovine or porcine corneas are used for comprehensive analyses of corneal penetration properties of therapeutic substances.^{45, 46} The drug development process is hampered by the limited throughput and high cost of *ex-vivo* and *in-vivo* animal models. These models demand prolonged experimental durations and substantial financial investments, impeding the streamlined assessment of novel drug candidates. Consequently, this not only extends the timeline for drug development but also imposes significant financial burdens on pharmaceutical enterprises.^{47, 48} Under the FDA Modernization Act 2.0, the FDA no longer requires new therapeutics to be tested on animal models. However, comparable data from alternative testing platforms is desired.⁴⁹ While this change marks a significant milestone in the transition away from animal testing, it also highlights the need to develop and validate physiologically

relevant *in-vitro* ocular models that could expedite the research and development process. Furthermore, such a development should increase the success rate of therapeutics entering the market while saving cost, time, and money.¹²

1.1.4 Improved cytotoxicity testing models address the limitations in current preclinical testing methods

The eye is an intricate organ with various protective barriers., including the conjunctiva, cornea, blood-aqueous and blood-retinal barriers, and efficient mechanisms like blinking and tear turnover that instantaneously remove therapeutics from the ocular surface.⁵⁰ This complex anatomy makes it very challenging to predict the toxicity of ophthalmic therapeutics in preclinical studies.⁵¹ To better determine the ocular toxicity and minimize the usage of animal models, the creation of sophisticated *in-vitro* ocular models that are physiologically relevant is necessary.^{52, 53}

There have been attempts to develop reconstructed human corneal epithelium-like models consisting of epithelial, stroma, and endothelial cell layers, such as EpiOcular™, Labcyte Cornea model, and MCTT HCE™, that are used to assess ocular irritancy and offer a more realistic and accurate alternative to current techniques.⁵⁴ Recently, Yu et al. developed a microfluidic chip using 3D printing, where they created a physiologically relevant eye model by culturing human corneal and conjunctival cells and introduced a controllable blink mechanism with tear flow mimicking the “eye”.⁵⁵ By controlling the blink rate, they introduced a dry eye state in the model and tested it with the introduction of a lubricant protein called Lubricin.⁵⁵ The development of 3D human cornea-equivalent models has been facilitated by rapidly advancing technologies, such as microfluidics and 3D printing.⁵⁶ However, the general adoption of these models can be constrained by expensive and tedious fabrication methodologies. The fabrication process can be challenging for other

laboratories to replicate, which can limit the widespread adoption and use of these models.⁵⁷ Despite these challenges, the continued development of these models holds great promise for the evaluation of drugs.⁵⁸

1.2 Overview of microfluidics

Microfluidics is the science and technology of fabricating devices that can be used to manipulate fluids at a microscale level, with channel dimensions ranging from tens to several hundred micrometres.⁵⁹⁻⁶¹ The movement of fluids at this microscale level is governed by different physical factors compared to the macroscale level.⁶² These principles include laminar flow, surface and interfacial tension, diffusion, capillary forces, and surface area to volume ratio.^{63, 64}

Laminar flow: The resistance for fluid to flow is higher in microchannels when compared to larger channels. This fluid behaviour is attributable to the fact that the thickness of the fluid (viscous force) has a more significant impact on the flow than the force driving it (inertial force).^{63, 64} Hence, the fluid flow is laminar (Reynold's number < 2000), which means the fluid travels smoothly or in a regular path that allows for easy manipulation of fluids.⁶⁴

Surface and interfacial tension: Surface tension arises due to the interaction of a liquid with air, while interfacial tension results due to the interaction of a liquid with another liquid. This property is utilized in cell sorting applications.^{62, 63}

Diffusion: Diffusion plays a vital role in separation processes in microfluidics. This process involves the random movement of particles from a higher concentration to a lower concentration. This process can be employed to create concentration gradients as the diffusion time is shorter in a microchannel environment.⁶³

Capillary forces: The capillary action involves the movement of fluid in a porous material or capillaries without the need for any external forces.⁶⁵ The fluid movement occurs due to surface tension and the wettability of capillaries.⁶⁵ These forces are more dominant than gravitational force and are employed in glucose monitoring kits.⁶⁴

Surface area to volume ratio (SAV): Microfluidic devices possess microscale channels, which result in higher surface area when compared to the volume, with surface forces being predominant.⁶⁶ For instance, a microfluidic chip with a channel dimension of 1 mm length x 100 μm width x 100 μm height has a total volume of 10 nL. The surface area of the channel walls would be 0.4 mm^2 , while the volume would be 10 nL or 0.01 mm^3 .⁶³ These characteristics can be utilized in digital microfluidic technology, where microdroplets are generated and transported through surface tension and substrate hydrophobicity, allowing for precise control and the potential to create a lab-on-a-chip.⁶⁶

1.2.1 Origin of microfluidics

The origin of microfluidics was influenced by four core fields, namely molecular biology, molecular analysis, biodefense, and microelectronics.⁵⁹ Firstly, it began with the miniaturization of chemical analytical methods, such as gas-phase chromatography and capillary electrophoresis, in the 1970s.^{59, 67} Secondly, after the end of the Cold War, the Defence Advanced Research Project Agency initiated the development of microfluidic systems that could be deployed in fields to detect chemical and biological threats in the 1990s.⁵⁹ Thirdly, with the rapid growth in the field of genomics in the late 1990s and early 2000s, microfluidics offered new innovative ways to overcome the challenges in the field of molecular biology, such as higher sensitivity and resolution for high-throughput DNA sequencing.⁵⁹ Lastly, the fabrication techniques of microelectronics, such as photolithography, were applied in microfluidics

to develop smaller lab-on-a-chip devices with efficient pumps and valves.⁵⁹ These advantages make the lab-on-a-chip devices an ideal platform for point-of-care diagnostics, drug discovery, biotechnology, analytical chemistry, energy generation, pharmaceuticals, and biomedicine.⁶⁷

1.2.2 Advantages of microfluidics

The advantages of microfluidics are as follows:

- i. Enables high-throughput processing, which makes it possible to execute several thousands of reactions simultaneously.⁶⁸
- ii. Replace cumbersome and expensive laboratory equipment necessary to execute samples with a tiny, portable device.⁶⁹
- iii. Minimizes the utilization of huge volumes of expensive reagents and samples used.⁶²
- iv. Provides a platform for integrating numerous laboratory processes onto a single chip, enabling enhanced diagnostics, personalized medicine, and point-of-care testing.⁶⁹

1.2.3 Disadvantages of microfluidics

- i. Microfluidic systems often require specialized knowledge and expertise in design, fabrication, and operation, which can be a barrier for researchers and industries without the necessary skills.⁷⁰
- ii. Microchannels in microfluidic devices are susceptible to clogging, which can disrupt experiments.⁷¹

- iii. Lack of standardization in microfluidic designs and fabrication methods can make it challenging to compare results across different studies or laboratories.⁷²

1.2.4 Types of microfluidics

A typical microfluidic system is composed of an inlet, outlet, microchannels, mixing chamber, and valves to regulate fluid flow (Figure 1.2:A).⁷³ Based on the device configuration, microfluidics can be classified as droplet-based microfluidics, paper-based microfluidics, digital microfluidics, and organ-on-a-chip microfluidics.

Droplet-based microfluidics: This involves forming several thousands of uniform microdroplets by utilizing the property of two immiscible fluids, such as water in oil droplets or oil in water droplets.^{74, 75} Microdroplets are generated at the interface of two immiscible phases. (Figure 1.2:B).⁷³ Each microdroplet serves as a reservoir that could be tailored for drug delivery, cell sorting, nanoparticle synthesis, and genomic analysis. This has emerged as a powerful tool, enabling researchers to perform complex assays with high accuracy and efficiency.^{73, 74}

Paper-based microfluidics: Paper is the most widely used material in paper-based microfluidics.⁷³ Cellulose and nitrocellulose-based porous materials are generally used. Paper is relatively hydrophilic, and the hydrophobic barrier can be created by techniques such as inkjet printing, wax printing, etching, and embossing. This patterning of hydrophilic and hydrophobic barriers allows the movement of fluids (reagents or samples) via capillary action (Figure 1.2:C).^{76, 77} This has gained much attention recently due to its low cost, ease of fabrication, portability, and versatility.^{73, 77} It is primarily employed in point-of-care (POC) diagnostics in the detection of pathogens, pollutants, pesticides, and nucleic acid based tests.⁷⁶

Digital microfluidics: This entails handling discrete liquid droplets (microlitre to picolitre) on a chip containing an array of electrodes (microelectronic chip) instead of a microchannel with other regions coated with a hydrophobic material. The individual droplet movements are controlled through electric fields instead of the network of channels, microvalves, pumps, and mixers.⁷⁸ It has applications in various disciplines, including biology, chemistry, medicine, and chemical engineering, and is widely utilized for genomic and proteomic analysis.⁷⁹

Organ-on-a-chip microfluidics: As the name suggests, organ-on-a-chip is a micro-engineered 3D *in-vitro* tissue model that could mimic the physiological conditions of human organ systems (Figure 1.2:D).⁸⁰ It aids majorly in the understanding of pathogenesis, organ-organ interactions, drug screening, and toxicology testing in a highly controlled environment.^{73, 80} It can serve as a link between preclinical and clinical trials, reducing the cost, time and accelerating the drug development process.⁷³

Based on the fluid flow incorporated into the aforementioned microfluidic devices, they can be categorized as active and passive.⁶⁸

Active microfluidics: This requires an external power source for fluid transport. These power sources include electroosmotic, peristaltic, and centrifugal or magnetic pumps. It is quite challenging and expensive to incorporate these external power sources into microfluidic chips due to the complexity involved in their miniaturization.^{68, 81}

Passive microfluidics: This employs fluid movement through inherent fluid properties, such as capillary action or surface tension, without needing any external

source. Since the fabrication process is simple and straightforward, requires less expertise, low cost, and is compact, this method is frequently used.⁶⁸

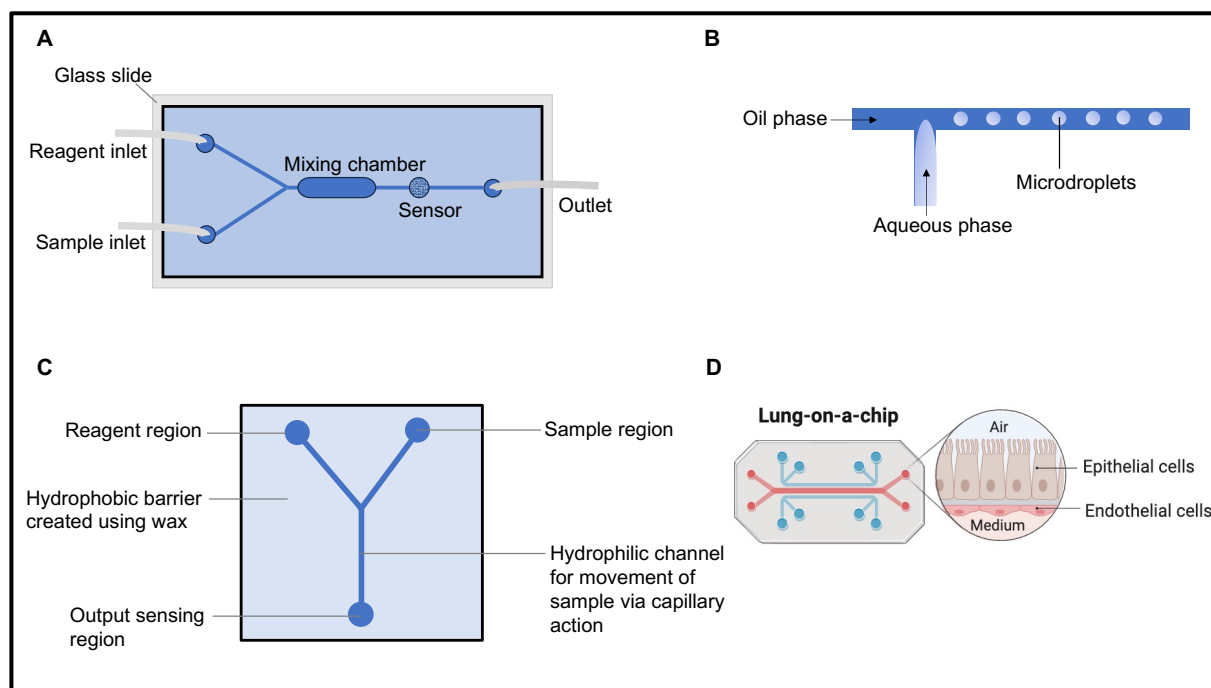


Figure 1.2: A) Simple microfluidic chip design B) Droplet-based microfluidics C) Paper-based microfluidics D) Organ-on-a-chip microfluidics.
(Created with Biorender.com)

1.2.5 Materials used for microfluidic fabrication

The materials used to fabricate microfluidic devices can be classified primarily as inorganic and organic.

Inorganic materials are chemical compounds that do not primarily consist of carbon-hydrogen bonds. The first series of microfluidic devices were manufactured using inorganic materials such as silicon or glass. Glass is optically transparent, while silicon is optically opaque.⁸² Si-glass microfluidic chips were developed initially for gas chromatography. They were commonly used due to their compatibility with a wide range of fluids, ability to generate highly precise microchannel features, and high

thermal conductivity.⁸³ They are majorly used in lab-on-a-chip devices integrating several components required to process an experiment in a single chip, especially for point-of-care diagnostics. It is also utilized in biochemical analysis and biosensing for DNA profiling, food safety testing, and environmental research.⁸⁴ However, their fabrication process is quite challenging, involving techniques such as photolithography and direct laser writing, which are highly expensive, time-consuming, labour-intensive, and use toxic chemicals.⁸² Additionally, silicon and glass are impermeable to gases, making it challenging to perform cell studies for longer durations.⁸⁵

Organic materials are chemical compounds primarily composed of carbon, hydrogen, and other elements such as oxygen, nitrogen, silicon, and sulphur. The fabrication process is more time-efficient and cost-effective for organic materials than inorganic materials. The organic materials used in microfluidic fabrication can be classified as thermosets, thermoplastics, hydrogels, and elastomers.⁸⁶

Thermosets are a class of polymers that undergo a permanent chemical crosslinking reaction when exposed to heat or radiation, forming three-dimensional structures. These materials cannot be melted or reshaped once cured. These materials provide high stability at high temperatures and are resistant to most solvents, making them ideal for applications requiring elevated performance.⁸⁷ They include materials such as SU-8 and polyimide, commonly used in lithography. Their material properties allow the creation of high-resolution microchannel features. Nonetheless, their application in microfluidics is limited by their high stiffness and high fabrication cost. Additionally, it's challenging to modify the surface of thermosets once cured, and bonding them can be difficult.^{82, 86}

Thermoplastics differ from thermosets; they become soft when heated and return to a solid state when cooled. This property allows them to be melted and

reshaped multiple times. They become malleable at their glass transition temperature (T_g) and can be manufactured using thermal moulding, producing thousands of replicates rapidly and affordably.⁸⁶ Moreover, the thermal moulding process is more affordable in industries as it is produced in high volume and is not suitable for prototyping. The emerging technologies, such as 3D printing and laser cutting, led to a significant increase in the rapid prototyping of thermoplastics. Some thermoplastics include polymethylmethacrylate (PMMA), polyethylene terephthalate, and polystyrene.^{82, 86} These materials are used to create lab-on-a-chip devices for applications like medical diagnostics and point-of-care testing due to their ease of fabrication and transparency, enabling real-time observation of fluid behaviour. Additionally, it is employed to design microfluidic pumps and valves for precise fluid control in various microscale experiments. The bonding of thermoplastics with other materials can be quite challenging, and often adhesive-based or thermal bonding is employed.^{86, 88} Despite their compatibility with a wide range of solvents, their usefulness in cell studies is limited since they are barely permeable to gases such as oxygen and nitrogen.^{82, 89}

Hydrogels are 3D networks of polymeric chains that span in aqueous media and are highly porous with tunable pore sizes.⁹⁰ These hydrogel-based microfluidic systems are particularly valuable in cell biology and tissue engineering, where they can mimic the physiological conditions of living tissues. They allow for precise control of factors like pH, temperature, and chemical gradients, making them ideal for studying cell behaviour, drug screening, and regenerative medicine applications.⁸² These systems are also employed in biosensors, where hydrogels can detect biological components, enhancing sensitivity and selectivity.⁹¹ The main disadvantage of hydrogels in microfluidics is their potential mechanical and structural instability,

especially in high-pressure microfluidic applications. Additionally, these hydrogels can undergo degradation with time, affecting their long-term functionality in microfluidic devices.⁹²

Elastomers are a network of crosslinked polymer chains that can be elongated or squeezed by an external force and regain their original shape on withdrawal of the force.⁸² Polydimethylsiloxane (PDMS) is the most extensively used silicone-based elastomer and a crucial component in microfluidics fabrication.^{93, 94} PDMS offers greater flexibility, biocompatibility, high optical transparency, gas permeability, water impermeability, thermal stability, versatile chemistry functionalization, and easy mouldability, making it ideal for biological applications.⁹⁵⁻⁹⁷ The liquid PDMS prepolymer and curing agent can be thermally cured in master moulds with nanometer resolution, and its low surface tension enables easy removal from the moulds.⁹⁴ The resulting PDMS can be bonded to another PDMS, polymeric materials (such as polyethylene terephthalate, polycarbonate, polyvinylchloride, PMMA), or glass/silicon substrates by reversible or irreversible bonding methods.^{94, 95, 98} PDMS being flexible allows the integration of other components such as electrodes, sensors, and valves, enabling the fabrication of multifunctional microfluidic devices. Moreover, the surface of PDMS is hydrophobic and requires surface modification to incorporate cell culture. Furthermore, the absorption of small hydrophobic molecules on its surface can limit its applications in certain biological analyses.⁹⁹

1.2.6 PDMS bonding strategies

PDMS can be easily attached to silicon/glass via surface activation techniques such as oxygen plasma treatment and corona discharge treatment. These techniques potentially alter the surface characteristics of silicon/glass and PDMS, enabling strong bonding between them.⁹⁴ PDMS can be bonded with thermoplastics by surface

functionalization, referred to as chemical gluing. In addition, it can be reversibly bonded by adhesives (Table 1.3).⁹⁴

1.2.7 Soft lithography – the most common PDMS fabrication method

The term soft lithography can be split into soft + lithography. The term “soft” refers to the flexible and elastic materials that can be fabricated using the soft lithography technique. “Lithography” is a general term used in microfabrication to create intricate surface patterns. Soft lithography is the modification of traditional photolithography techniques. In photolithography, light creates tiny patterns on silicon substrates for making rigid computer chips and other electronic devices. In the context of soft lithography, the term implies a similar process of pattern transfer but with a focus on using flexible materials as substrates.^{100, 101}

The process begins with the creation of a master mould using traditional photolithography. Here, a negative photoresist material is spin-coated over the silicon wafer.⁹⁵ Photoresists are light-sensitive materials used to engrave patterns on silicon substrates. They can be classified as positive and negative photoresists. On exposure to UV light, positive photoresists become soluble while negative photoresists become hardened. Typically, SU-8, a negative photoresist material, is spin-coated over silicon wafers due to its thermal stability, excellent adhesion, sensitivity to UV light, and biocompatibility.¹⁰² Then, UV light is projected through the photomask, a specialized template that exposes only the region of photoresists to be hardened by UV light. The part of photoresist material exposed to UV light can be hardened, and other regions are dissolved away. The hardened material bearing the pattern results in the reverse master mould.¹⁰³ A liquid precursor, generally a PDMS prepolymer and curing agent mixture (Sylgard 184 from Dow corning), is poured over the master mould, cured at elevated temperatures, and peeled off to produce a PDMS stamp with the desired

structures.^{97, 99, 100} Additionally, this reusable PDMS stamp (which can be used > 50 times) can act as a template to cast different polymeric materials. The properties of PDMS, such as low interfacial free energy (~ 21.6 dyn/cm) and inert surface, make it ideal for casting different polymeric materials without adhesion.⁹⁷ There are a few constraints in using PDMS stamps, such as $\sim 1\%$ shrinkage on curing, and the height/length aspect ratio cannot be too low or high as they might result in structural defects due to PDMS softness.⁹⁷

Soft lithography encompasses other techniques, namely replica moulding, microcontact printing, injection moulding, and hot embossing.⁹⁷ In replica moulding, an already existing master mould is utilized to cast UV or thermally curable prepolymers that replicate the mould's shape and morphology.¹⁰⁴ A PDMS stamp or mould is fabricated via soft lithography in microcontact printing. The surface of the PDMS stamp is then inked with a polymeric material, which is often self-assembling monolayers (SAM) (alkane thiolates on Au/Ag and alkyl siloxanes on hydroxyl-terminated surfaces such as Si/SiO₂). These SAMs can selectively stick to the substrate when the stamp comes into contact with the surface, enabling the transfer of the desired pattern to the substrate.⁹⁷ In injection moulding, thermoplastic prepolymers are extruded from the funnel-shaped container and directed into a heated barrel. Subsequently, they are propelled under pressure into the heated mould cavity. The temperature is maintained below the glass transition point, resulting in the solidification of mould.¹⁰⁴ Hot embossing, similar to injection moulding, utilizes pressure and heat to shape the mould and transfer the pattern directly to polymeric substrates instead of injecting the material into a cavity.¹⁰⁴

These methods generate high-resolution replicas (< 100 nm), which enables rapid fabrication compared to photolithography, precision patterning, and versatile

material selection.^{97, 104} However, mass manufacturing is restricted by the need for expensive equipment, it is a labour-intensive and time-consuming process, requires a semi-clean room fabrication, resulting in increased cost, and it is limited to two-dimensional (2D) structures.¹⁰⁴ These challenges could be overcome by exploiting 3D printing for microfluidic fabrication.⁵⁶

Table 1.3 - Comparison of PDMS bonding techniques

Bonding techniques	Description	Bonding strength	Advantages	Disadvantages
Oxygen plasma treatment	In a plasma chamber, the surface of PDMS is oxidized by replacing the terminal methyl groups ($-\text{CH}_3$) with repeating units of silanol groups ($-\text{O}-\text{Si}(\text{CH}_3)_2-$)	Strong covalent bonding and can withstand pressure between 180 kPa and 715 kPa	Uniform and consistent activation of PDMS surface	High-end expensive equipment should be bonded immediately after plasma treatment as they regain hydrophobicity in ambient conditions due to the reorientation of polar groups and require clean room fabrication
Corona discharge treatment	The tip of the corona treater device generates high potential that ionizes the surrounding air, creating a localized plasma that activates the PDMS surface	Relatively less bond strength when compared with oxygen plasma treatment (227 - 380 kPa)	Inexpensive, portable device, easy to use	The device is operated manually and mainly depends on the sample and treatment height, speed, and time, which results in inconsistent activation of the PDMS surface

Chemical based gluing	Organosilanes such as APTES ((3-aminopropyl) triethoxysilane) are used as a chemical glue to functionalize the PDMS surface by reaction between the silanol group of PDMS and the amine group of APTES	184 kPa	No requirement for specialized equipment or expertise	Some adhesives comprise volatile organic compounds that are hazardous and require safe handling
Adhesive based gluing	PDMS can be bonded by the application of silicone or epoxy-based adhesives	Bond strength is weaker when compared to chemical gluing	No requirement for specialized equipment or expertise	Often used in combination with chemical-based gluing to improve bond strength ^{94, 105-107}

1.3 3D printing – an emerging technology for rapid prototyping

3D printing has emerged as a cost-effective alternative for rapidly prototyping microfluidic devices.^{58, 108} 3D printing is an additive manufacturing technology that systematically fabricates 3D objects layer by layer.^{96, 109} The advantages of 3D printing are low cost, time efficiency, a simplified process compared with a conventional method such as soft lithography, a wide range of material availability, and reduced labour.^{58, 96} The process of 3D printing can be described as follows, which includes modelling, printing, and post-processing (Figure 1.3):

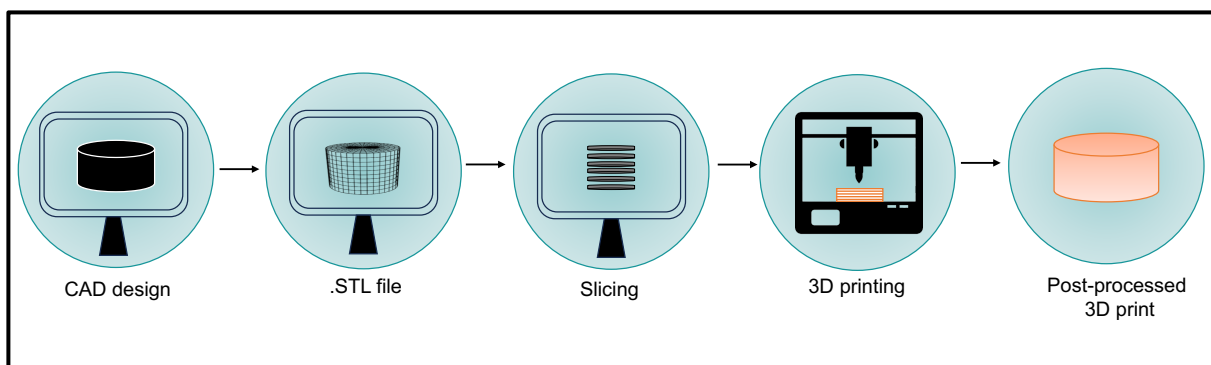


Figure 1.3: 3D printing process

- i. The design to be 3D printed can be created using computer-aided design (CAD) software or by a reverse engineering technique in which an existing 3D object can be scanned utilizing a 3D scanner.¹¹⁰
- ii. The designed file can be saved in .stl (Standard Tessellation Language) file format.¹¹¹
- iii. The object in the .stl file can be sliced into multiple layers depending on the lowest Z dimension height specified utilizing 3D printing preprocessing open source software such as chitubox.¹¹²

- iv. The sliced file can be fed as input into any 3D printer, and the 3D printer prints the object layer by layer, resulting in the final 3D print.^{112, 113}
- v. The 3D printed object is post-processed (isopropyl alcohol washing, UV curing, sanding) depending upon its applications.¹¹²

1.3.1 Types of 3D printing

Extrusion-based printing, inkjet-based printing, and vat-based printing are the most commonly used 3D printing methods for microfluidic fabrication (Figure 1.4).^{96, 114, 115}

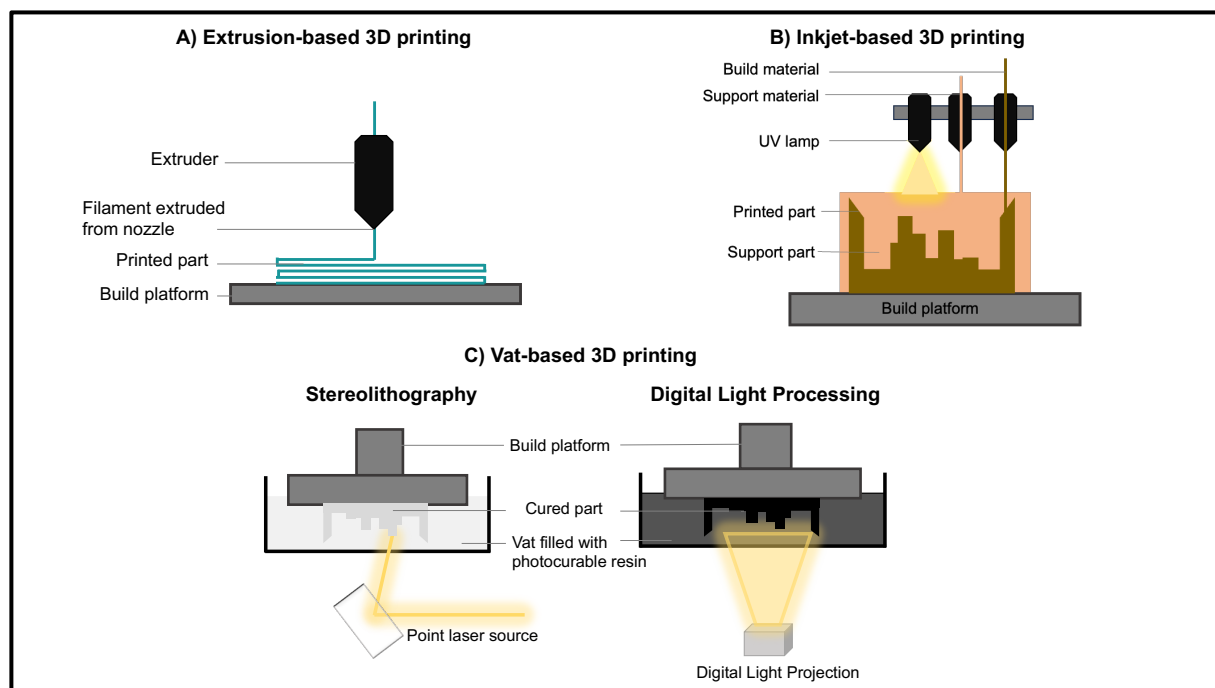


Figure 1.4: Types of 3D printers: A) Extrusion-based B) Inkjet-based C) Vat-based

1.3.1.1 Extrusion-based 3D printing

Extrusion-based methods fall into two broad categories: filament-based and non-filament-based.¹¹⁵ Fused Deposition Modeling (FDM) is the most commonly used filament-based 3D printer. FDM utilizes polymers in filament form, while the non-

filament-based approach uses powder-based materials and hydrogels.¹¹⁵ The FDM method is relatively simple and can be accessed easily due to its low cost.⁹³ In this method, biocompatible and inexpensive thermoplastic polymer materials like polylactic acid, PMMA, thermoplastic polyurethane, and polypropylene are extruded from the motor-driven nozzle that can move in three directions point by point, thereby constructing the entire device (Figure 1.4:A).^{96, 115, 116} At the nozzle, the thermoplastic polymer is heated above its melting point to melt the polymer. When extruded, the polymer becomes solidified at room temperature.¹¹⁵ There are several challenges in using FDM, as the printed devices are either opaque or semi-transparent and lack structural integrity, as the sealing between the layers is not strong, making it unsuitable for microfluidic device fabrication.⁵⁸

The introduction of the "3D bioplotter" in 2002 marked the launch of the first 3D printer that did not depend on an extrusion-based technique.¹¹⁷ It operates on the same principle as FDM but focuses primarily on bioprinting applications employing cell-laden bioinks or hydrogels.¹¹⁷ It also features heating and cooling systems to prevent cell damage, and pneumatic pressure drives the extrusion of bioinks.^{115, 117} Wang et al. conducted a study using the FDM method to develop a 3D printed corneal scaffold using Gelatin Methacrylate (GelMA) combined with Hyaluronic acid. This study aimed to create a scaffold that could potentially aid corneal transplant procedures.¹¹⁸ While FDM excels in producing cost-effective and accessible parts for general manufacturing and prototyping, the "3D bioplotter" stands out in specialized biomedical applications, particularly in bioprinting tissues, scaffolds, and cell-laden structures, offering a more targeted solution for advanced medical research and therapeutic interventions.⁹³

1.3.1.2 Inkjet-based 3D printing

In inkjet-based printing, there are two primary methods: photopolymer and powder-based techniques. The photopolymer approach is often referred to as MultiJet Modeling (MJM).¹¹⁵ In MJM, the curable liquid photopolymer is printed layer by layer on a built platform, where the printhead has multiple nozzles and is cured on exposure to a UV lamp (Figure 1.4:B).¹¹⁹ The support materials for 3D prints are made of wax that can be dissolved away after printing.¹¹⁵ This technology also employs a computer-assisted array of inkjet heads for controlling the volume of photopolymer droplets ejected.¹²⁰ The advantage of MJM is that numerous materials with different properties (like hard and soft plastic) can be extruded simultaneously.¹¹⁹ The high resolution and multi-print capability make it ideal for microfluidics applications.⁹⁶ The disadvantages of MJM are that the material formulations are expensive, and studies regarding the suitability for biological applications and bioactivity of the materials are absent.⁹⁶

In powder-based inkjet printing, the build platform is coated with a layer of powder material, mainly metallic and ceramic powders.¹¹⁵ The inkjet head extrudes an aqueous binding agent in a precise manner layer by layer to form the final object. The support material is also made of powder, which can be removed easily after 3D printing and does not require postprocessing steps.⁹⁶

1.3.1.3 Vat-based 3D printing

This consists of a vat filled with photocurable polymers. A build platform is lowered into the vat, and a photopolymer gets cured on selective exposure to a light source (380 – 405 nm). The build platform moves up, the second layer gets selectively cured, and the entire object is formed.^{115, 121} Stereolithography (SLA) and Digital Light Processing (DLP) are the most commonly used vat polymerization methods.^{121, 122}

Charles W. Hull invented SLA in 1984, the first commercial 3D printing technology.¹¹⁷ The difference between SLA and DLP lies in the type of laser source employed. SLA utilizes a point source laser beam of a very small diameter, which cures the resin point by point at each layer, resulting in higher resolution, with the trade-off of longer print duration than DLP.¹²³ DLP is a recently emerged and advanced projector-based technology. It uses digital micromirror device-based projectors (a dynamic mask with thousands of rapidly rotating micromirrors that reflect light) to project a light source that cures the entire resin layer at once. Thus, DLP prints are significantly faster than SLA-based methods.^{115, 123}

The quality of the 3D print mainly depends upon the light source, resin material, vat tank, and build platform used.¹²¹ There are a variety of commercially available resins for vat-based printing. These resins are generally made of acrylate monomers, acrylate oligomers (crosslinking agents), photoinitiators, plasticizers, and photo-absorbers.¹²⁴ The photoinitiators are activated by a light source, producing free radicals. These free radicals react with monomers and oligomers, forming a 3D network of polymers.¹²⁵ In addition, plasticizers and other additives are added to the resin formulation to modify the 3D print's flexibility, tensile strength, and visual appearance based on its intended use. SLA and DLP methods offer high resolution and optically translucent prints at a lower cost, and high throughput can be achieved in terms of printing speed and resolution. These advantages make it an ideal choice for microfluidic fabrication,¹²⁶ and these are the 3D printers used in the research described in subsequent experimental chapters in this thesis.

1.3.2 3D printing fabrication approaches in microfluidics

The microfluidic chip fabrication employing 3D printers can be split into two categories: direct 3D printing and indirect 3D printing.^{115, 127}

Direct 3D printing, commonly known as the "one-step fabrication strategy," is one of the quickest fabrication techniques.¹¹⁵ Here, microfluidic chips are 3D printed, encompassing microchannels and other components such as valves, pumps, inlets, outlets, and reaction mixers without further bonding or assembly.^{115, 127} Additionally, the 3D printed device might require post-processing steps to improve its biocompatibility, optical transparency, and surface smoothness.¹¹⁵ Despite its reported advantages, it is limited by suitable material availability and dimension accuracy, and it is difficult to 3D print intricate geometries as it can be affected by resin blockage.¹²⁸

Indirect 3D printing, commonly known as the "mould-based approach," combines 3D printing and conventional casting.^{115, 129} The process begins with the fabrication of a 3D-printed mould, similar to the soft lithography technique.¹²⁹ These 3D-printed moulds may be sacrificial or non-sacrificial.¹²⁸ After the moulds are 3D printed, PDMS is cast and thermally cured. Regarding non-sacrificial moulds, PDMS is peeled off from the mould and subsequently bonded to another PDMS structure or glass by permanent or temporary bonding strategies.⁹⁴ In the case of sacrificial moulds, the final step is the dissolving of sacrificial components. Consequently, these sacrificial moulds can be utilized once, while non-sacrificial moulds can be used multiple times.¹¹⁵ The indirect 3D printing approach can circumvent the shortcomings of direct 3D printing, such as gas impermeability, cytotoxicity, and low optical clarity.¹¹⁵ Further, this method does not require multiple processing steps involved in soft lithography and can be fabricated rapidly at a low cost.⁵⁸

1.3.3 Challenges in PDMS fabrication from 3D printed moulds

The high cost associated with PDMS chip fabrication is the main hindrance to microfluidics use amongst researchers worldwide.⁶² However, with the advancements in 3D printing technology and the availability of affordable commercial 3D printers that

offer high resolution, the future holds promising.⁵⁸ Despite these developments, the fabrication of a PDMS chip from a 3D-printed mould presents a myriad of challenges, notably while using vat-based 3D printers.⁶²

- i. **Poor surface quality:** Typically, the surfaces of 3D-printed moulds are extremely rough.¹²⁴ This results in an inconsistent surface finish of PDMS chips when cast on these 3D printed moulds.¹¹⁵ Moreover, the uneven surface of the PDMS can affect its bonding with other materials and its optical transparency.¹¹⁵
- ii. **Low optical transmittance:** Optical clarity is one of the distinguishing features of PDMS that makes it ideal for imaging and cell applications.¹³⁰ However, the uneven surface of PDMS cast in 3D printed mould results in optically translucent PDMS chips.¹³¹
- iii. **PDMS curing inhibition and lack of resin compatibility:** The uncured resin monomers and photoinitiators on the mould surface act as catalyst poison, inhibiting the PDMS curing process.^{62, 132} This results in a sticky PDMS chip, with additional and unwanted PDMS adhered to the mould surface.⁶²

Researchers have made several attempts to fabricate smooth, completely cured PDMS devices from 3D printed moulds. Comina et al. coated a 3D printed mould with specialized ink via air brushing to prevent PDMS curing inhibition. However, air brushing requires extensive practice, and coating thickness is crucial in this process.¹³³ Waheed et al., UV cured the 3D printed mould for 5 minutes, followed by soaking in ethanol for 6 hours. Then, the mould was subjected to plasma treatment for a minute and silanized by perfluorooctyl triethoxysilane for 3 hours to obtain fully cured and smooth PDMS.¹³⁴ Chande et al. spin-coated the 3D printed mould with SU-8, a

negative photoresist commonly used in soft lithography to prevent PDMS curing inhibition.¹³⁵ Villegas et al. coated the 3D printed mould with omniphobic lubricant coatings that generated smooth PDMS.⁹⁸ Moreover, the silanizing agents and photoresist materials mentioned above are toxic, and coating them on 3D printed moulds can be time-consuming and requires high-end, expensive equipment.^{98, 135} Furthermore, there is limited research on producing optically transparent and smooth PDMS chips utilizing inexpensive 3D printers that can be used for cell applications.

1.4 Revamping cell culture studies using the PDMS chip platform

In-vitro cell culture studies play a crucial role in cell biology research. These studies involve culturing cells in controlled external environmental conditions to understand better cell behaviour, cell-cell interactions, and the response of cells to physical or chemical stimuli.¹³⁰ Additionally, *in-vitro* cell-based HTS of drugs is essential for identifying the lead compound and determining drug toxicity in the preclinical stages.¹³⁶

1.4.1 Static conventional cell culture and its limitations

Currently, in laboratories, the majority of cell culture studies are conducted in petri dishes or microtiter plates under static environmental conditions.^{137, 138} The cells used in cell culture can be categorized as finite, continuous/ immortal, and stem cell lines (Table 1.4). Primary cells are obtained from tissues or organs isolated from living organisms, including humans and animals. They can only be grown for a limited number of generations prior to senescence, the point at which they can no longer multiply.¹³⁹ Continuous cell lines, conversely, are derived from genetically modified cell lines, are immortal, and do not undergo contact inhibition.¹⁴⁰ Stem cells are undifferentiated cells obtained from multicellular organisms that can be differentiated

into a specific cell type. These three cell types can be adherent (polygonal epithelial cells, elongated fibroblast cells) or free-floating suspensions. Suspension cells are easier to handle than adherent cells, as the latter involves trypsinization to remove the cells adhered to cell culture plates.^{139, 140}

Cell culture studies involve a series of steps beginning with the isolation of cells from a tissue or cell source. The desired cell of interest can be isolated by enzymatic digestion using proteolytic enzymes, and the reaction is stopped utilizing enzymatic inhibitors. The cell isolation and subsequent seeding experiments are conducted in a laminar air flow hood under aseptic conditions.¹⁴¹ The isolated cells are then seeded onto a petri dish or a multiwell plate precoated with extracellular matrix proteins to promote cell adhesion and proliferation.¹⁴² The cells are cultivated in a specific nutritional medium that provides all essential nutrients and growth factors for cell growth. The most commonly used media are Dulbecco's Modified Eagle Medium (DMEM) and Roswell Park Memorial Institute (RPMI) media, composed of carbohydrates, amino acids, pH indicators, salts, and vitamins. Most basal medium, also known as serum-free medium, lacks proteins, growth factors, and lipids to avoid contamination. Hence, they require 5-20% fetal bovine serum supplementation and antibiotics (penicillin, amphotericin B, streptomycin).¹³⁹ The composition of the medium varies depending on the cell types and experimental conditions. The culturing conditions typically vary depending on the particular cell type being used.

For instance, for mammalian cell lines, the cells are observed under a microscope for their morphology and to ensure no contamination after seeding. Then, the cells are checked for viability and placed in a humidified CO₂ incubator at 37 °C to promote cell growth.^{141, 143} These cells are monitored for any contamination, change in cell morphology, and proliferation rate at regular intervals. In addition, the culture

medium is changed regularly to replenish nutrients and remove waste metabolites from cells. When the cells proliferate and reach 70 to 80 % confluency (no space for cells to grow as a monolayer), they are subcultured into a new multiwell plate, a process known as passaging.^{141, 143} After a series of passages, the grown cells can be utilized in experiments to determine the toxicity of various medications or materials.¹⁴⁴ Furthermore, cell lines derived from ocular tissues can serve as invaluable models for understanding eye diseases, screening potential drug candidates for conditions like glaucoma or retinal disorders, studying cellular responses to environmental factors affecting ocular health, and conducting genetic and molecular investigations to advance our knowledge of eye biology and vision science. They are also essential for developing treatments and therapies for various eye disorders, contributing to advancements in ophthalmology and vision-related healthcare.⁴³

The gold standard for evaluating the toxicity of newly developed therapeutic agents in preclinical studies involves testing in 2D cultured cells, which means growing cells on a flat surface.^{144, 145} Performing toxicity validation of therapeutics in 2D cell culture offers a more standardized protocol that allows greater experimental control and reproducibility, reduces the usage of animal models, and is a simple, cost-effective method with relatively lower maintenance requirements.¹⁴⁶ According to the ISO10993 standards, the biomedical implants or extracts from the implants are incubated with cultured cells to check for any cytotoxicity to ensure safety and biocompatibility for humans.¹⁴⁷ Despite these benefits, cells are cultivated under static environmental conditions using this method.¹⁴⁸ In practice, the cells are cultivated in the same medium for multiple days, resulting in depleted nutrients, accumulation of waste metabolites, altered cell physiology and viability, and stress-indicating factors. Furthermore, the extracellular microenvironment acidifies the medium within minutes.

In contrast, *in-vivo* cells are maintained under dynamic environmental conditions and are continuously perfused with extracellular fluid, providing a surplus of nutrients and removing metabolic waste simultaneously.¹⁴⁸ In addition, this approach requires a substantial amount of expensive chemicals and is susceptible to human error when handling several tens to hundreds of samples simultaneously. In recent years, these obstacles have prompted researchers to develop new, cost-effective techniques to culture cells in a dynamic environment, an improved *in-vitro* cell culture model.¹³⁰ Growing cells in a microfluidic environment is gaining popularity for these reasons.⁶⁰

Table 1.4 Comparison between primary, continuous, and stem cell lines

Properties	Primary cell lines	Continuous cell lines	Stem cell lines
Source of origin	Human tissues or organs	Established cell lines obtained from the Animal Tissue Culture Collection	Derived from human embryos or tissues and reprogrammed cells
Biological relevance	High	Low	High
Consistency	Medium	High	Variable depending on the stem cells used
Genetic integrity	Maintains its genetic information	Prone to genetic drift	Prone to genetic and epigenetic changes
Usability	Requires optimized culture conditions	Well-standardized conditions and protocols	Requires specialized techniques and protocols for maintenance and differentiation
Cost and time	Higher cost as slower cell growth rate	Lower cost as faster cell growth rate	Higher cost and longer time due to the complex nature and specialized culture requirements of stem cells. ¹⁴⁸⁻

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1.4.2 Dynamic cell culture studies using PDMS chips

PDMS-based microfluidic chips are gaining increased attention for the creation of sophisticated *in-vitro* cell culture models due to their distinctive features such as optical transparency, gas permeability, and easy mouldability.^{60, 151} In addition, the precise control over small volumes of fluids, high throughput analysis, automated processes, and real-time monitoring of cell culture parameters through the integration of sensors make it appropriate for cell applications.¹³⁸ Furthermore, other characteristics, such as reduced reagent consumption (which decreases experimental cost and time), continuous nutrient supply, and simultaneous removal of waste metabolites, are significant advantages.¹³⁸ Despite these benefits, the inherent high surface hydrophobicity of PDMS poses a considerable obstacle to the incorporation of cells in PDMS-based systems.¹⁵¹

1.4.2.1 Surface treatment of PDMS for improved cell culture

PDMS hydrophobicity is mainly attributed to the presence of organic methyl groups within its structure. The hydrophobicity of PDMS leads to low surface wettability on the PDMS surface, leading to poor cell adhesion and proliferation. Wettability is the ability of a liquid to spread over the surface and is typically determined by measuring the water contact angle (WCA). The WCA of PDMS is generally $108^\circ \pm 7^\circ$ ($> 90^\circ$ is considered a highly hydrophobic surface), resulting in poor cell attachment.^{99, 152} The surface of PDMS is rendered hydrophilic via several techniques, including plasma treatment with a biomaterial coating and various chemical modifications.¹⁵²

1.4.2.1.1 Plasma treatment with biomaterial coatings

This is the most prevalent method for modifying the surface of PDMS. The surface of PDMS is exposed to dissociated forms of gases, including nitrogen,

hydrogen bromide, chlorine, and argon, with oxygen being the most commonly used. When reacting with the PDMS surface, these gas molecules replace the organic methyl groups with hydrophilic silanol groups via oxidation.¹⁵² The effectiveness of this technique is constrained by the quick hydrophobic restoration of PDMS upon exposure to air, typically within an hour.¹⁵² This technique can enhance the adhesion of other coating materials for prolonged cell adhesion.¹⁵³

After plasma treatment, the PDMS surface can be coated with various extracellular matrix (ECM) proteins, including collagen, gelatin, laminin, and fibronectin. These proteins are natural substrates that enhance cell attachment and promote cell survival on the PDMS surface.^{151, 152, 154-156} These proteins can promote cell attachment and govern diverse cellular functions, including orientation, morphology, proliferation, migration, gene expression, and functionality. Collagen, particularly collagen type I, is typically utilized due to its high adsorption and ability to increase the hydrophilicity of PDMS.^{156, 157} Gelatin, a collagen derivative, is a thermo-responsive hydrogel that facilitates cell adhesion and stability under shear stress.¹⁵⁸ Fibronectin is well-known for its high rate of protein adsorption and distinct interactions with cell membrane receptors, inducing robust cell adhesion.¹⁵³ Other biopolymers, such as laminin, have also been researched; however, their use is much lower.¹⁵² Although ECM proteins facilitate cell attachment, the long-term development of cells can be hindered by the dissociation of cells and cell cluster formation.¹⁵² Consequently, the ECM protein coating preferred depends upon the cell type and intended experimental duration.^{152, 153}

1.4.2.1.2 Chemical surface modification

Chemically modified PDMS surfaces provide strong surface adhesion for cells, and these non-organic reagents do not degrade, providing advantages over

techniques employing ECM proteins.¹⁵³ One of these non-organic reagents is the silica-titania composite, composed of silicon dioxide and titanium dioxide.¹⁵⁹ Silica-titania, such as methyltriethoxysilane and tetraethylorthosilane, are utilized for PDMS surface modifications in various combinations. They form a thin SiO₂ layer on the PDMS surface, facilitating greater cell adhesion. Additionally, silane coupling agents such as 3-aminopropyltriethoxysilane (APTES) act as an anchor to immobilize other biomolecules. APTES forms amine (NH₂) groups on the PDMS surface, increasing its hydrophilicity and forming interactions with other biomolecules.¹⁵² Silanization of PDMS using APTES resulted in a self-assembling monolayer of protein molecules that supported improved cell proliferation and attachment even under stressful conditions.¹⁶⁰ However, APTES is carcinogenic and is not suitable for long-term investigations.^{123,125,161} Furthermore, plasma treatment of PDMS is required prior to the application of the silane-based coating, which requires high-end equipment not accessible to everyone.¹⁵³ Polyethylene glycol (PEG), a synthetic amphiphilic polymer, can act as an intermediate between the hydrophobic PDMS surface and the surrounding environment, projecting its hydrophilic group for cell adhesion. PEG reduces the WCA of PDMS to 57°, increasing the hydrophilicity of PDMS.¹⁵² However, PEG-coated PDMS exhibited poor cell adhesion akin to an untreated PDMS surface.^{152, 162}

Polydopamine (PDA), a synthetic polymeric material, was first synthesized from a protein-based adhesive identified in mussels' feet.^{163, 164} Biocompatibility, hydrophilicity, cell adhesiveness, and functionalization of other biomolecules via Schiff base reaction are some distinctive properties of PDA.¹⁶³ Furthermore, PDA can transform diverse hydrophobic surfaces (including ceramics, polymers, and metals) into hydrophilic surfaces with long-term stability.¹⁶⁴ The adhesive nature can be

attributed to the catechol group and amine group present in the chemical structure.¹⁵⁷ Under alkaline conditions, dopamine self-polymerizes to form PDA, establishing a strong covalent interaction with the hydrophobic surface.¹⁵² Several researchers have cultivated stem cells on PDA-coated PDMS chips. Yang et al. cultured human umbilical vein endothelial cells in a cylindrical PDMS microchannel coated with PDA, which mimics the human blood vessel. This work contributes to the development of platforms that more accurately simulate *in-vivo* conditions.¹⁶⁴

1.4.3 PDMS chips – versatile tools for cell applications

PDMS chips for cell applications are gaining attention due to technological advancements in other disciplines, such as cell biology, biomaterials engineering, drug delivery, and molecular biology.⁸⁰

Bai et al. developed a novel cornea-on-a-chip using the corneal epithelial/endothelial cells isolated from mice. This device allows a coculture between epithelial and endothelial cells, separated by a collagen scaffold.¹⁶⁵ The collagen scaffold resembles the bowman layer, a dense layer of collagen fibers that supports the cornea, positioned between the epithelial and stromal layers of the eye.¹⁶⁶ This in-vitro system replicates the basic corneal structure and can be used as a model to examine the penetration of topical medications.¹⁶⁵

Abdalkader et al. developed another cornea-on-a-chip model that accounts for the shear stress during blinking. The chip included corneal epithelial cells cultivated in the porous membrane, separating the top and lower channels. Bidirectional and continuous flow was induced on the top and bottom surfaces of the cells, respectively.¹⁶⁷

Chung et al. developed an eye-on-a-chip model of the retinal pigment epithelium/choroid complex to examine choroidal neovascularization, a crucial phase in numerous eye disorders that lead to blindness. This model demonstrated the pathogenesis of choroidal neovascularization, which was alleviated using the drug bevacizumab. This eye-on-a-chip model provides scope for progressing *in vivo* testing of ocular therapeutics and supplementing animal models in research and development.¹⁶⁸

1.4.4 Future direction: advancing ocular drug testing using PDMS chips

In recent years, the increased prevalence of eye-related illnesses attributable to rapid changes in lifestyle and an aging population has highlighted the need for effective ocular therapies.³ Most ocular therapeutics fail in clinical trials due to unforeseen drug toxicity, leading pharmaceutical companies to incur significant losses in both money and time.¹⁶⁹ Nevertheless, the discipline is transforming with the integration of microfluidics, broadening possibilities for creating sophisticated *in-vitro* ocular models. This innovative approach bridges the gap between preclinical investigations and human clinical trials.¹⁷⁰

Moreover, creating a cost-effective fabrication process that renders this platform accessible to all scientists across the globe continues to be a significant impediment. Understanding these limitations, researchers are harnessing the potential of 3D printing technology to develop affordable and scalable fabrication processes. This innovation would facilitate access to advance *in-vitro* ocular models, encouraging collaboration and accelerating the drug development process.^{3, 43, 58}

The future looks promising with emerging technologies, including 3D printing and artificial intelligence.¹⁷¹⁻¹⁷³ By harnessing these technologies, it is possible to

envision a future where the ocular drug development process becomes more efficient, cost-effective, and accessible, ultimately benefiting patients worldwide.

2 Thesis rationale

According to the WHO, there are currently 2.2 billion individuals globally experiencing visual impairment.¹⁷⁴ With the increase in the prevalence of eye-related disorders such as glaucoma, diabetic retinopathy, cataract, and age-related macular degeneration, the global ophthalmic drug market is forecast to grow from \$13.3 billion in 2022 to \$23.4 billion in 2030.¹⁷⁵ This emphasizes the necessity for effective intervention and treatment strategies.¹⁷⁵ Alarming, the majority of drug candidates fail clinical trials, primarily attributable to unanticipated cytotoxicity in preclinical investigations.¹⁶⁹ Typically, in laboratory investigations, most cytotoxicity investigations are conducted in a static, 2D cell culture environment.¹⁷⁶ Despite the standardization of the evaluation of therapeutics for toxic effects in cell culture plates, this approach can be rather time-consuming, arduous, and costly, as well as challenging to implement high throughput screening and automation.¹⁴⁸

To overcome these hurdles, a shift towards dynamic, sophisticated *in-vitro* ocular models is of paramount importance. These constraints can be circumvented by employing microfluidic technology, which delivers dynamic environmental conditions for cell growth through an automated approach.⁶⁰ PDMS is the most highly regarded elastomeric material in microfluidics for cell applications due to its unique features, such as biocompatibility, oxygen permeability, and optical transparency.¹³⁰ Despite the enticing properties of PDMS, its widespread implementation is constrained by time-consuming, cumbersome, and unaffordable conventional fabrication procedures such as soft lithography.⁵⁸

3D printing, an emerging rapid prototyping technique, is gaining tremendous popularity for fabricating microfluidic chips.⁵⁸ 3D printing offers numerous benefits,

including low cost, time efficiency, a simplified process compared to soft lithography methods, a wide variety of material availability, and minimized labour.⁵⁸ Despite its tremendous advantages, creating PDMS from 3D-printed moulds presents numerous obstacles. Firstly, unreacted resin monomer on the mould surface hinders the PDMS curing process.⁶² Secondly, the rough surface of the mould produces PDMS chips that are optically translucent.⁹⁸ Thirdly, the hydrophobic nature of the PDMS surface hinders adequate cell adhesion.¹⁵²

This thesis aims to utilize 3D printing to fabricate PDMS chips for ocular cell applications. The first experimental chapter (Chapter 3) focuses on the fabrication method for optically transparent PDMS chips using 3D printed moulds. This chapter tackles the difficulties in producing PDMS chips from 3D printed moulds by introducing simple post-processing steps involving heating, sanding, and chemical coating. Here, the hydrophobic PDMS surface can be rendered hydrophilic by coating PDMS chips with PDA, a polymer that self-polymerizes in alkaline conditions and requires no specialized equipment.¹⁵⁷ The direct implication of this chapter's findings is that the PDMS chip can be produced at a low cost and made accessible across all research labs.

The second experimental chapter (Chapter 4) discusses the integration of ocular cells into the PDMS chip fabricated from post-processed 3D printed moulds. PDA-coated PDMS chips can be used to incorporate HCEC under dynamic environmental circumstances. The outcome of this chapter is a low-cost, high-throughput approach for culturing cells on PDMS chips, which can serve as a cornerstone for the creation of inexpensive ocular disease models for drug development. The final chapter (Chapter 5) summarizes the results and discusses the potential application of HCEC chip platforms in toxicity studies.

3 Inexpensive and Rapid Fabrication of PDMS Microfluidic Devices for Biological Testing Applications Using Low Cost Commercially Available 3D Printers

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This chapter has been published as follows:

Ramasamy, Megala, Brandon Ho, Chau-Minh Phan, Ning Qin, Carolyn L. Ren, and Lyndon Jones. "Inexpensive and rapid fabrication of PDMS microfluidic devices for biological testing applications using low cost commercially available 3D printers." *Journal of Micromechanics and Microengineering* (2023).

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Author	Concept/Design	Data Acquisition	Analysis	Write up/Publication
Ramasamy	Y	Y	Y	Y
Ho	Y	Y	Y	Y
Phan	Y			Y
Qin	Y			
Ren	Y			Y
Jones	Y			Y

3.1 Abstract

Polydimethylsiloxane (PDMS) elastomers have been extensively used in the development of microfluidic devices, capable of miniaturizing biomolecular and cellular assays to the microliter and nanoliter range, thereby increasing the throughput of experimentation. PDMS has been widely used due to its optical clarity and biocompatibility, among other desirable physical and chemical properties. Despite the widespread use of PDMS in microfluidic devices, the fabrication process typically requires specialized facilities, instruments, and materials only available in a limited number of laboratories. To expand microfluidic research capabilities to a greater scientific population, we developed and characterized a simple and robust method of fabricating relatively inexpensive PDMS microfluidic devices using readily available reagents and commercially available 3D printers. The moulds produced from the 3D printers resolve designed microfluidic channel features accurately with high resolution ($>100\text{ }\mu\text{m}$). The critical physical and chemical post-processing modifications we outline here are required to generate functional and optically clear microfluidic devices.

3.2 Introduction

The process of drug and cellular research and development involves several phases, beginning with pre-discovery to mechanistic studies and ending with clinically validated compounds. Common methods for these kinds of discovery programs, especially in the early phase, often include high-throughput liquid handling and screening systems, automated robots, and sophisticated software for analyses. These assays can be complicated and expensive, making it difficult for researchers outside of well-funded industrial or academic settings to adopt these technologies. Additionally, performing chemical screens with conventional multi-well plate-based assays can consume precious and expensive biological samples or compounds. As a result, there has been explosive growth in microfluidic devices, commonly referred to as lab-on-a-chip (LOC) concepts, with wide-ranging applications designed for simple use, yet still performing complex assays. Microfluidic devices can manipulate fluids at the microscale with laminar fluid flow, providing an extremely high degree of control for tuning the microenvironment for cell culture.^{130, 177, 178} The ease of use for these devices, in addition to the miniaturization of biochemical and cellular assays, serves to increase assay throughput that can be easily performed by all researchers. These advantages make it an ideal platform in various disciplines, such as drug discovery, biosensors, point-of-care diagnostics, analytical chemistry, energy generation, pharmaceuticals, and tissue engineering.^{104, 133, 179}

PDMS is the most widely used silicone elastomer for microfluidic device fabrication due to its many appropriate properties, including flexibility, biocompatibility, high optical transparency, and gas permeability, among others.¹⁷⁹⁻¹⁸² Due to these properties, particularly optical transparency, PDMS microfluidic devices have been widely used in microbiology and mammalian cell-based assays to observe the

response of cells to various environmental stresses through single-cell imaging.^{130, 182,}
¹⁸³ The conventional method of PDMS chip fabrication involves a soft lithography process, wherein SU-8 negative photoresist material is spin-coated over the silicon wafer followed by UV exposure via a photomask to create the protrusions of a desired pattern on the microfluidic master mould.^{107, 184} The resulting master mould produced via this method has a superior resolution ($< 20\ \mu\text{m}$) and acts as a counter-mold for the PDMS polymer. Finally, a PDMS prepolymer mould is cast against the master with raised relief structures representing the designed fluidic network. Despite being a popular method for generating biocompatible PDMS devices, soft lithography can be time-consuming, labour-intensive, limited to 2D structures, highly expensive, and requires cleanroom fabrication.^{95, 135} In addition, it is very challenging to make 3D structures because it will involve multiple spin-coating and UV exposure processes.

Recent advancements in 3D printing technologies and the availability of low-cost 3D printers with high precision offer an alternative option for the rapid prototyping of an array of biomedical devices, notably microfluidic devices.^{133, 179, 185-187} Various types of 3D printing technologies exist in the biomedical field, including Fused Deposition Modeling (FDM) and Stereolithography (SLA). FDM is a common and inexpensive technique that deposits a thermoplastic filament onto the build platform, assembling the 3D object in layers, albeit at lower resolutions than other techniques. SLA is another widely used printing technique that uses high energy (UV) light to solidify photosensitive polymers in liquid form, also building layer-by-layer but at a greater resolution ($< 50\mu\text{m}$). Derivatives of SLA exist, including Masked Stereolithography Apparatus (MSLA), and Digital Light Processing (DLP), both of which use a light source that is selectively masked to create the 3D design, with the difference being the method by which the light source illuminates the resin.^{115, 121, 187}

Indeed, there has been increasing adoption of 3D printing methods to generate PDMS microfluidic devices in a similar fashion to well-established soft lithography processes, challenging the traditional methods for PDMS fabrication.⁵⁸ Researchers can design, 3D print, and test PDMS devices within days or even hours, enabling rapid prototyping.

Wide adoption of PDMS devices made using 3D printed masters still remains a challenge. This is likely due to the high cost and specialized training required to perform traditional PDMS manufacturing in a laboratory setting. Furthermore, there has been limited successful implementation of PDMS devices generated from 3D-printed moulds for biomedical research.^{188, 189} Two possible factors are the lower quality of the material surface and the resolution of the desired channel feature on the device compared to soft-lithography-based methods, which may deter researchers from 3D printing-based fabrication.^{96, 190} Therefore, the challenge remains to readily fabricate these devices accurately and inexpensively. Here, we describe a simple, rapid, and inexpensive method to create PDMS microfluidic devices using commercially available 3D SLA printers. We characterize the synthesized devices and recommend print settings and parameters to provide the most accurate microfluidic features. Finally, we report on a novel post-processing step that generates optically clear PDMS devices, an important feature for biological and imaging applications. Most importantly, our method utilizes reagents that can be found and/or purchased readily in all research laboratories and requires no specialized training. Altogether, our work makes 3D printing and microfluidics techniques more feasible for all researchers across many disciplines.

3.3 Materials and Methods

3.3.1 Design of Microfluidic Devices

All microfluidic devices used in this study were designed using the open-source 3D modelling software FreeCAD (<https://www.freecadweb.org/>, version 0.19.0). Designs of all microfluidic devices can be found in supplementary files. All stereolithography (.STL) design files generated in FreeCAD were then loaded onto the 3D printers for device fabrication.

3.3.2 Fabrication of 3D printed master-moulds

Master moulds to cast PDMS microfluidic chips were fabricated using one of the following commercial stereolithography (SLA) 3D printers: AnyCubic Photon D2 DLP (AnyCubic, Guangdong, China) or Form 3B Low Force Stereolithography™ (Formlabs Inc. PKG-F3B-WSVC-MSP-BASIC, Somerville, MA, USA).

For 3D printed moulds using Form 3B, models were printed with clear resin (Clear V4 Resin, Formlabs Inc., RS-F2-GPCL-04, MA, USA) at a minimum resolution of 25 μm , following manufacturer recommended print settings using PreForm software version 3.28.0 (Formlabs Inc., Somerville, MA, USA). For 3D printed counter-moulds using the AnyCubic Photon D2 DLP, models were printed with black resin (AnyCubic, SKU TL4425, Guangdong, China) at a minimum resolution of 51 μm , following manufacturer recommended print settings using Chitubox software v.1.9.4 (Chitubox Inc, Guangdong, China).

3.3.3 Post-processing of the 3D printed master-mould

3.3.3.1 UV and heat treatment of mould

The master-moulds completed printing on their respective printers. The moulds were carefully removed from the build plate using a metal wedge and rinsed in 100% isopropanol (IPA) for a maximum of 30 minutes at room temperature. The 3D printed moulds were washed in 100% isopropanol with gentle agitation for a maximum of 30 minutes, air dried, and UV cured at 60°C for 30 minutes using the Form Cure, UV curing device (FormLabs Inc. FH-CU-01, Somerville, MA, USA). These UV treated moulds from both SLA printers were heated at 100 °C for 0, 2, and/or 4 hours, depending on the experiment.

3.3.3.2 Sanding and nail polish coating of mould

Following UV and heat treatment, moulds from both SLA 3D printers were subjected to several rounds of sanding with increasing grit sandpaper. First, moulds were smoothed with 1500 grit sandpaper, next with 3000, then 7000, and finally 15,000 grit sandpaper. A thin layer of nail polish (NP) was subsequently coated on the sanded surface and heated at 75 °C for 30 minutes.

3.3.4 Fabrication and post-processing of PDMS microfluidic devices

Polydimethylsiloxane (PDMS) (Sylgard 184, Dow Corning, Copley, OH, USA) prepolymer and curing agent were mixed in a 10:1 ratio and centrifuged at 500 g for 10 minutes. The centrifuged PDMS mixture was cast into post processed 3D printed moulds obtained from both Form 3B and AnyCubic printers and allowed to cure at room temperature in the fume hood for 12 hours to allow for bubbles to dissipate. The moulds containing PDMS were then incubated at 75 °C for 1.5 hours. Finally, they were removed from the mould using a metal scalpel. Inlet and outlet holes were

generated in the resultant PDMS device using a hole puncher (UniCore Manual Punchers, QIAGEN WB10028, Germantown, MD, USA). Finally, the PDMS microfluidic device was adhered to a glass microscope slide (VWR 100491-372, Radnor, PA, USA) using medical grade double-sided tape (ARcare 90106NB, Little Rock, AR, USA). For experiments measuring the bond between PDMS-glass surfaces, PDMS devices, and glass microscope slides were bonded using oxygen plasma. The adhered device was baked at 95°C for 10 minutes.

Post-processing of the final PDMS devices was performed using a coating solution containing polydopamine (PDA) to increase surface hydrophilicity. Coating solutions were prepared by dissolving dopamine hydrochloride (Sigma-Aldrich H8502, St. Louis, MO, USA) in phosphate buffered saline (PBS) pH 8.5. PDMS of dimensions 20 x 20 x 5 mm (length x width x height) were dipped and submerged in 0.1% or 0.01% PDA coating solution for 2, 8, and 24 hours with gentle shaking at room temperature. Finally, they were washed with distilled water and air dried in a fume hood.

3.3.5 Brightfield Microscopy

Following complete curing, the PDMS microfluidic devices were imaged using brightfield microscopy (Zeiss Axiovert 25 CFL, Carl Zeiss Inc., Oberkochen, Baden-Württemberg, Germany) to visualize the device features (microfluidic channels and wells) and characterize the device for potential defects. Images were acquired and processed using AxioVision (Carl Zeiss Inc.) and ImageJ computer software (<https://imagej.nih.gov/ij/download.html>, National Institutes of Health (NIH), Bethesda, MD, USA).

3.3.6 Optical transmittance of PDMS devices

The optical transmittance of PDMS microfluidic devices cast and released from 3D printed moulds was measured in the visible spectrum between 400 - 700 nm using the Imaging Multimode Plate Reader (Cytation 5, Agilent BioTek Instruments, Winooski, VT, USA). Small PDMS circular cut-outs of approximately 22 mm in diameter with 5 mm thickness were placed in 12-well tissue culture plates (Montreal Biotech Inc. MBI703001C, Dorval, QC, Canada) and then scanned in the plate reader. As a positive control, PDMS was cast and cured in a 12-well plate.

3.3.7 Microfluidic assembly with fluid flow in a sample PDMS device

Metal 20-gauge blunt tip needles (HaBeuniver HB13510ML921DZT, Fuzhou, Fujian, China) were connected to FEP tubing (Masterflex® Transfer Tubing, VWR MFLX06406-60, Radnor, PA, USA) and then connected to the PDMS microfluidic devices at inlet and outlet positions. We created a PDMS device as a “test” case that contains three 5 cm long rectangular shaped channels (1 mm wide and 0.5 mm deep) in parallel (CAD file available upon request) to perform fluid flow experiments, and this device served as our test case. For all experimentation with fluid flow, a commercial syringe pump (PHD ULTRA, Harvard Apparatus, Holliston, MA, USA) was used to infuse the microfluidic chip with distilled water. Food colouring dyes (red, blue, and yellow) were added to distilled water to create coloured solutions for easier fluid flow visualization through the entire sealed microfluidic system. Syringe pumps were programmed to generate flow rates at minimum and maximum rates of 0.5 $\mu\text{L}/\text{min}$ and 100 $\mu\text{L}/\text{min}$, respectively, for a period of 24 hours.

3.3.8 Estimation of shear stresses

High shear stresses can negatively impact cellular proliferation and viability. Low and non-lethal shear stresses must be achieved in order to use microfluidic devices for cell-based assays. To determine the theoretical minimum and maximum shear stresses in our system, The flow rates within the microfluidic device were converted to shear stress values using an online tool provided by Darwin Microfluidics, Microfluidic Flow Rate and Shear Stress Calculator (<https://darwin-microfluidics.com/blogs/tools/microfluidic-flowrate-and-shear-stress-calculator>, Darwin Microfluidics, Paris, Île-de-France, France).

3.3.9 Sessile drop water contact angle (WCA) measurements

PDMS is hydrophobic naturally. To evaluate the uniformity of the fabricated PDMS chip surface, the contact angle of five positions of each PDMS device was measured using an Optical Contact Analyzer (DataPhysics Instruments GmbH, Filderstadt, Germany) to determine the contact angle, using a sessile drop technique. In this method, 5 μL of high-performance liquid chromatography (HPLC) grade water was deposited on the PDMS surface at a rate of 2 $\mu\text{L/s}$ and imaged once a droplet settled and formed. The water contact angles of the resulting images were analyzed using SCA 20 software (version 2.04, Build 4). For each replicate, a minimum of five separate droplets were quantified across the surface of the PDMS device, and the reported values are an average of these independent measurements.

3.3.10 Tensile strength measurement between PDMS and glass

PDMS-glass bonded devices were anchored to a spring balance (Ajax Scientific ME495-2000, Toronto, Canada), and a force was applied to the PDMS

device, separating the PDMS from the glass slide. The force (in Newtons) at which the PDMS peeled from the glass surface was recorded.

3.4 Results

3.4.1 Commercial 3D printers can readily assist in the fabrication of PDMS microfluidic devices

We set out to utilize commercial 3D printing, an emerging technique used in the milli- and micro-fluidic field, as a tool to design PDMS microfluidic devices and characterize critical processing steps and printing considerations that result in optically clear PDMS microfluidic chips. Positive master moulds were printed using FormLabs 3B+ and AnyCubic Photon D2 and were subjected to various post-processing steps to generate a smooth 3D printed part (Figure 3.1a). These processing steps involved high heat treatment, physical sanding, and applying a chemical coat. The resulting moulds were used to cure and create optically clear PDMS (Figure 3.1b). In our test case involving three separate channels (dimensions: 100 μm height x 1 mm width x 3 cm length; volume of 3 μL), we were able to flow dyed solutions with rates as low as 0.5 $\mu\text{L}/\text{min}$ and as high as 100 $\mu\text{L}/\text{min}$ (Figure 3.1b). This corresponds to a minimum and maximum of 0.083 mm/s and ~16.7 mm/s, respectively, equating to shear stresses between 4.5 – 891 Pa. Therefore, despite opting to use an adhesive rather than the conventional and stronger plasma bonding approach for sealing the device, our system can withstand high pressures. Furthermore, by selecting the proper media flow rates, we can achieve low shear stresses that have been demonstrated to be non-lethal to many mammalian cell lines.¹⁹¹ Nonetheless, it is likely this can be improved through improved channel dimensions.

Next, we sought to determine the accuracy of the commercial SLA printers in printing the CAD-designed moulds, and how well this translated to the final PDMS device. SEM imaging of positive moulds and PDMS devices generated by FormLabs and AnyCubic printers confirmed that the designed channel features could be resolved (Figure 3.1c, 3.1d). Notably, both commercial printers could create channel heights as low as 100 μm , with the FormLabs capable of going as low as 50 μm (Figure 3.1c). However, at <100 μm , the channel features were less resolved, and both the 3D print and resultant PDMS device did not form sharp, distinct walls and instead were slanted (Figure 3.1c, 3.1d). To quantify this, we measured the angle between the channel wall and a theoretical plane perpendicular to the printed surface, which would otherwise be a perfect wall if the printer replicated the CAD design perfectly (Figure 3.1e). Indeed, we found that only designed channel heights >200 μm resulted in more accurately printed channel walls (<10 degrees deviation) (Figure 3.1e). In the XY plane, we found that the AnyCubic printer was able to accurately fabricate the desired channel widths. Surprisingly, the FormLabs printer consistently created channel widths that were ~ 100 μm greater than the CAD design (Figure 3.1f), a factor that must be considered in 3D design. Nevertheless, with proper 3D design and an understanding of the limitations of each printing method, we showed that commercial 3D printing can certainly be used in the design and fabrication of PDMS microfluidic devices.

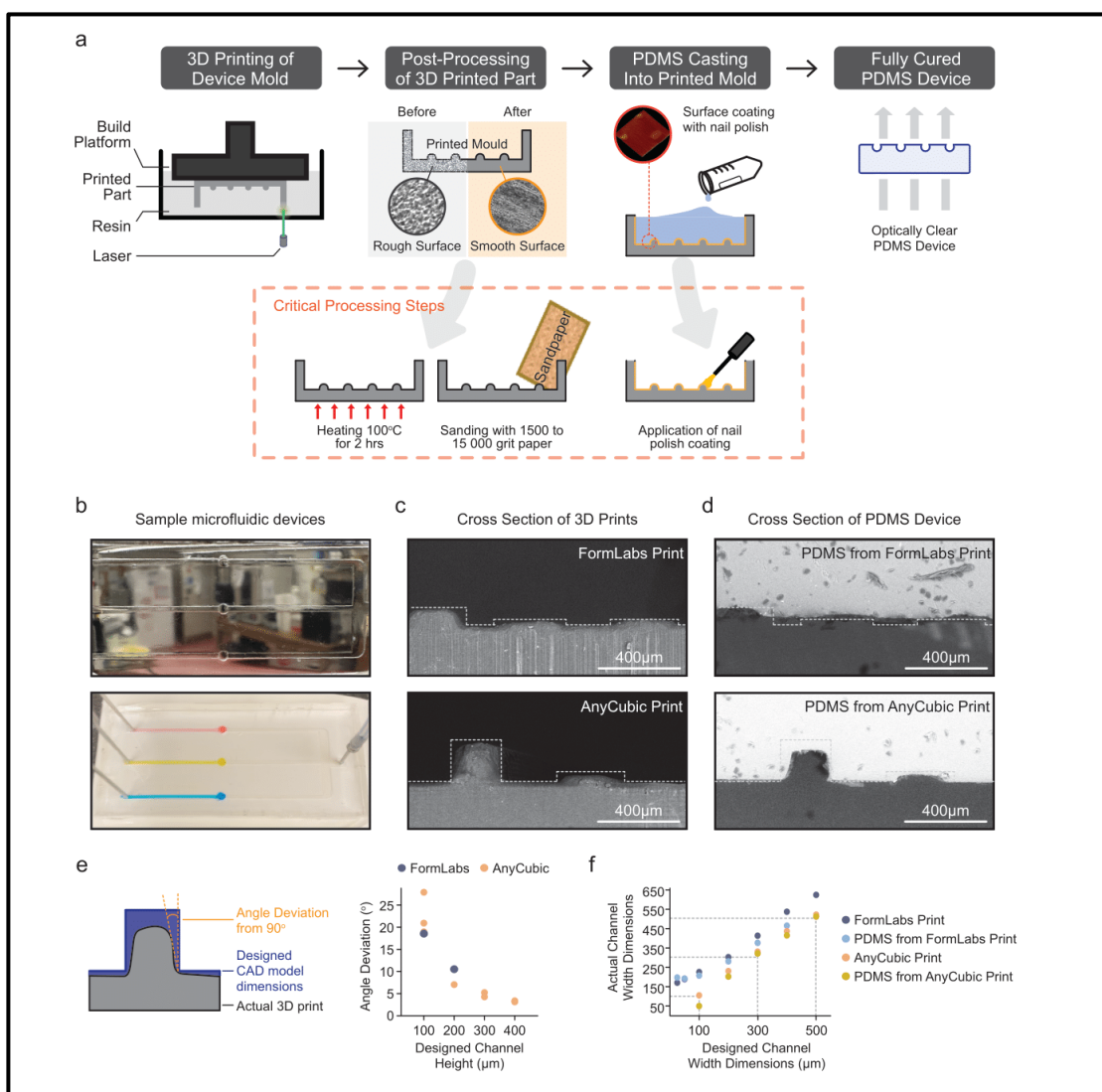


Figure 3.1: Commercial 3D printing is a power tool in the fabrication of PDMS microfluidic devices.

(a) Outline of the fabrication and post-processing method. Master moulds were 3D printed using the Formlabs Form 3B using clear resin or the AnyCubic D2 DLP using black resin. 3D printed parts were post-processed, which involves critical steps including heat treatment, physical sanding, and chemical coating with nail polish. These post-processed moulds were used to cure PDMS, resulting in a fully cured, optically clear microfluidic device. **(b)** Sample images of the microfluidic device (top) adhered to a glass microscope slide using double-sided medical-grade tape and (bottom) to the double-sided tape alone with dyed distilled water flowing through. Cross sections of the **(c)** 3D printed mould or **(d)** the resulting PDMS device were imaged using a scanning electron microscope. Dotted lines represent the theoretical feature that should have

been printed and was designed in the CAD software. (e) Measurements of the angle between the hypothetical feature (channel wall) and the actual printed channel wall, as a proxy for accuracy of the 3D printed mould, for both FormLabs and AnyCubic printed parts. (f) Measurements of the channel features that resolved on the 3D printed mould compared to the designed width dimensions in the CAD file. Dotted grey lines are a reference for perfect printing; features that printed the same dimension as initially designed.

3.4.2 Heat treatment of 3D printed moulds required for PDMS curing

We observed a significant amount of PDMS curing inhibition at the interface between the PDMS surface and the 3D printed surface, which has been previously reported in applications involving 3D printed moulds (Figure 3.2). The resultant PDMS did not fully cure, leaving behind a sticky texture with many channel features failing to form, or being formed at very low resolution. To relieve this inhibition, we treated our printed moulds with high heat at 100°C for up to four hours. This resulted in a completely cured and translucent PDMS surface, through manual observation and brightfield microscopy (Figure 3.2). Notably, the surface of the device was smooth, and the device features were translated properly. We found that longer heat treatments resulted in increased PDMS curation, as evident by an improved PDMS surface texture and perfect channel edges. Importantly, at extremely high temperatures (~120°C) or prolonged heating (> 8 hrs), the 3D printed moulds warped and/or cracked, decreasing the likelihood of generating PDMS microfluidic devices with accurate channel dimensions (data not shown). Overall, 3D prints acquired from SLA printers require high heat treatment to allow complete PDMS curing.

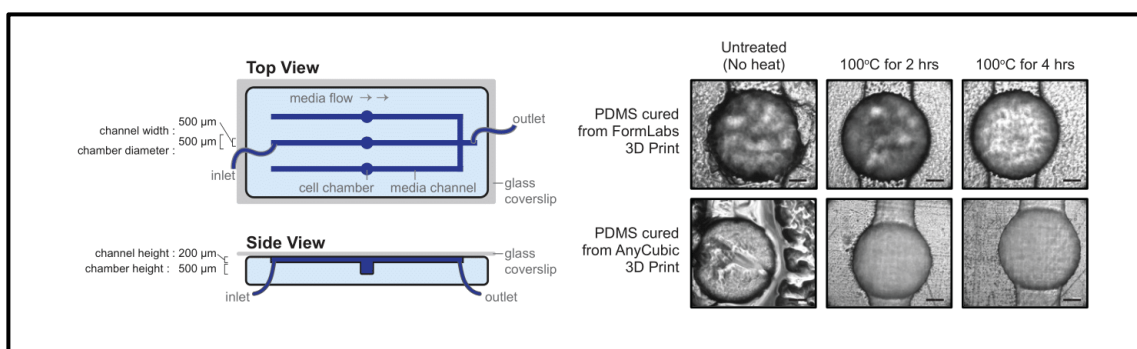


Figure 3.2: Heat processing is required for complete PDMS curing in 3D printed moulds.

(Left) A schematic representation of PDMS device, with arrows, indicated the direction of media flow through the channels and chambers within the device. **(Right)** Brightfield microscopic images of PDMS obtained after removal from the 3D printed mould that was subjected to varying high heat treatment durations. Scale bars indicate 100 μm .

3.4.3 Post-processing of 3D printed moulds is required for generating smooth PDMS surfaces

Despite the successful fabrication of a PDMS microfluidic device, the resultant PDMS devices were optically translucent, likely due to the surface roughness of the 3D printed moulds. The PDMS surface was textured, and not clear, microscopically (Figure 3.2). Furthermore, when visualizing the surface of the PDMS devices obtained using these moulds by brightfield microscopy at higher magnification (63X magnification), it was immediately clear that the surface was uneven and rough (Figure 3.3a). The surface contained various imperfections, such as streaks and ridges. Therefore, we sought to design an inexpensive and simple post-processing method that would dramatically reduce the surface roughness of our 3D prints, ideally translating to the PDMS device. Ultimately, our goal was to reduce surface roughness and imperfections on the PDMS device itself, as this is a critical requirement for optical clarity. We opted to sand the features of the 3D moulds with increasing grit sandpaper,

from 1,500 to 15,000 grit, to remove large debris and imperfections in the 3D print, which resulted in a visually smoother surface, but left streaks and scratches (Figure 3.3a). Therefore, with sanding alone, the resulting PDMS devices were translucent and conformed to the scratches visible on the moulds. Polishing and applying chemical-based coatings, such as spray painting and lacquer coatings, as a final surface finish, have been described.¹²⁴ We explored several chemical coating methods, including silicon-based sprays (mould release spray), polyvinyl alcohol (PVA), and polyurethane, all of which inhibited PDMS curing. However, we also assessed the ability of nail polish, an extremely common coating agent that is readily available, to smoothen the surface of the moulds. We noted that nail polish alone can reduce the surface roughness of the 3D prints, and significantly reduce the visible surface roughness when combined with initial sanding of the 3D print (Figure 3.3a). Both post-processing steps reduced the surface roughness of the 3D print by almost two-fold (root mean square roughness (Rq) from 119.16 to 63.23; Figure 3.3b) and were suitable to cure PDMS.

Next, we used atomic force microscopy (AFM) to obtain higher resolution images and quantified the Rq of the 3D printed moulds and resulting PDMS surfaces. Interestingly, PDMS devices fabricated from moulds that had not undergone post-processing exhibited lower surface roughness than its mould counterparts (FormLabs: Rq from ~ 119 to 99 nm; AnyCubic: Rq from ~ 259 to 171 nm). However, with post-processed moulds, there was an extreme reduction in surface roughness of the resulting PDMS device in all cases when compared to the non-processed PDMS device (FormLabs: Rq from ~ 63 to 18 nm; AnyCubic: PDMS device with Rq ~ 32.42 nm). We note that in all technical replicates of the AnyCubic 3D printed mould, we could not get reliable estimates of the surface roughness. Nevertheless, the resulting

PDMS surface obtained from the moulds from both commercial printers was > 5-fold smoother than PDMS obtained from an untreated mould.

The resultant PDMS surfaces obtained through post-processing exhibited greater clarity, visibly, compared to the non-processed counterpart (Figure 3.3c). This was further validated when we assessed the optical transmittance (a measure of clarity) of various post-processed PDMS samples, within the 400 – 700 nm range, compared to a control PDMS surface cured in a glass mould. We found that PDMS surfaces originating from moulds that were post-processed with both mechanical sanding and nail polish coating resulted in the highest level of transmission (78 – 83 %) across scanned wavelengths compared to the non-processed moulds and approached the transmission of the control PDMS surface, which exhibited transmission levels of 85 – 89 % (Figure 3.3c). Notably, the clarity of PDMS surfaces from moulds treated with only a nail polish coating was still quite clear (75 – 81 %). This suggests that this coating is the main factor for PDMS surface smoothness and clarity, with mechanical sanding responsible for increasing clarity by ~ 2 – 3 %, likely due to the removal of larger surface debris and imperfections. It should be noted that we observed that physical sanding can cause up to 50% removal of resin material that forms our microfluidic features, which is approximately ~ 40 μm change in height in our devices (Figure 3.3d). Additionally, applying nail polish was observed to add ~ 11 μm of height in our devices (Figure 3.3d). These two factors must be considered when performing these post-processing steps. Future work will expand on these methods to limit the amount of 3D printed material that is lost from sanding and optimizing the nail polish coating to minimize the additional material added to our master mould. Nevertheless, our data outline the importance of the post-processing of 3D printing moulds to generate optically clear PDMS devices.

One other potential issue with rough PDMS surfaces is its ability to be bonded to glass surfaces using oxygen plasma. A smoother surface could facilitate greater PDMS-glass bonding. To investigate this, we oxygen plasma bonded PDMS (1 cm x 1 cm x 1 cm cut outs) from moulds produced using the FormLabs printer, with and without nail polish treatment (Figure 3.3e). Surprisingly, we find that our PDMS substrates, regardless of nail polish treatment, are capable of bonding to glass tightly. When tensile stress was applied, commonly, the PDMS substrate would break internally before its bond with glass was broken (Figure 3.3f). The tensile force required to cause the PDMS or PDMS-glass bond was on average, 13.20 and 12.12 Newtons for substrates generated from untreated and nail polish-treated moulds, respectively (Figure 3.3g). This equates to 0.1320 and 0.1212 Megapascals, which is at least within an order of magnitude and corroborates other reported values.¹⁹² Therefore, PDMS surfaces derived from FormLabs-based master moulds can be oxygen plasma bonded, though a smoother surface may facilitate more even bonding through the microfluidic device.

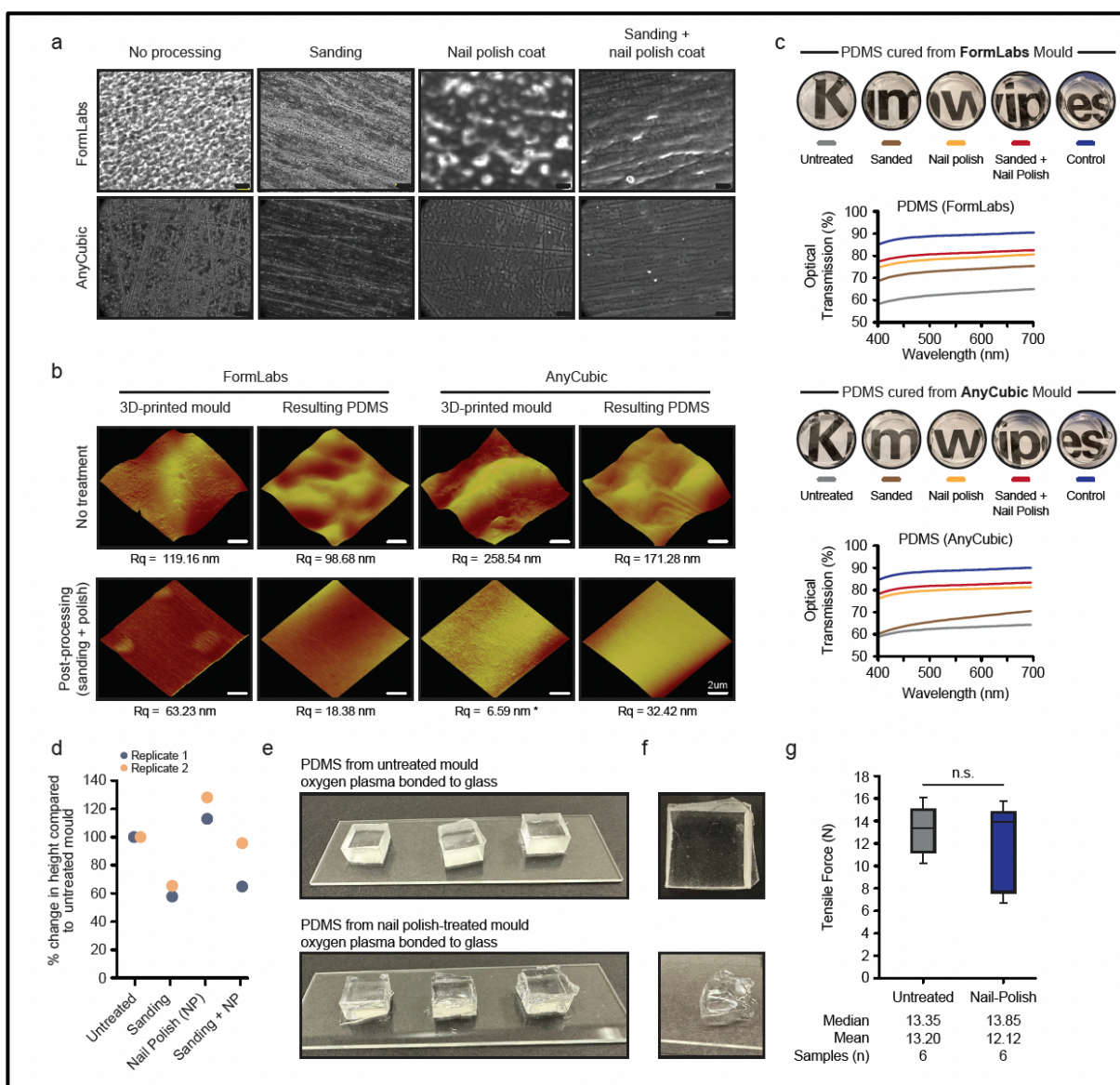


Figure 3.3: Sanding and chemical coating of the 3D print generates a smoother surface that results in optically clear PDMS devices.

(a) Brightfield images of PDMS surfaces obtained from FormLabs and AnyCubic 3D printed moulds after post processing steps. Scale bars represent 50 μm . (b) Three-dimensional AFM image of both the 3D printed surface and the PDMS surface obtained from each respective mould. The root mean square roughness (Rq) for each surface is indicated below the image. (c) Circular PDMS cut-outs were obtained from 3D printed moulds, either post-processed or not, and the optical transmission (%) was calculated and plotted for both FormLabs- and AnyCubic-derived PDMS surfaces. (d) Change in channel dimension of post-processed 3D printed moulds compared with untreated 3D printed moulds

(e) Square PDMS cut-outs (1 cm x 1 cm x 1 cm, lwd) were generated from 3D printed moulds, with or without nail polish treatment, and oxygen plasma bonded to glass slides. (f) Top-view of PDMS plasma bonded to glass (top) and an image of residual PDMS material still remaining after the force was applied to remove PDMS from glass slide (bottom). (g) The tensile force applied to remove PDMS from the glass slide was recorded using a spring scale. The median, mean, and number of samples are indicated below.

3.4.4 PDA- and collagen-coated PDMS devices have greater surface hydrophilicity

Despite being highly biocompatible, PDMS is also highly hydrophobic, which can pose significant complications for microfluidic devices intended for cell-based applications. Therefore, we applied a chemical surface modification (previously reported to increase surface hydrophilicity) using polydopamine (PDA).⁹⁶ PDMS substrates were coated with various concentrations of PDA for 2, 8, or 24 hours, and the water contact angle (WCA) was measured as a proxy for surface hydrophilicity. PDA-coated devices coated exhibited significantly reduced WCA compared to non-coated PDMS surfaces (Figure 3.4). Notably, this effect was concentration dependent as surfaces coated with 0.1% PDA had a smaller WCA than surfaces coated with 0.01% PDA. Therefore, the resultant PDMS microfluidic devices fabricated using our can be modified similarly to those reported in the literature to produce hydrophilic surfaces that are suitable for cell culture applications.

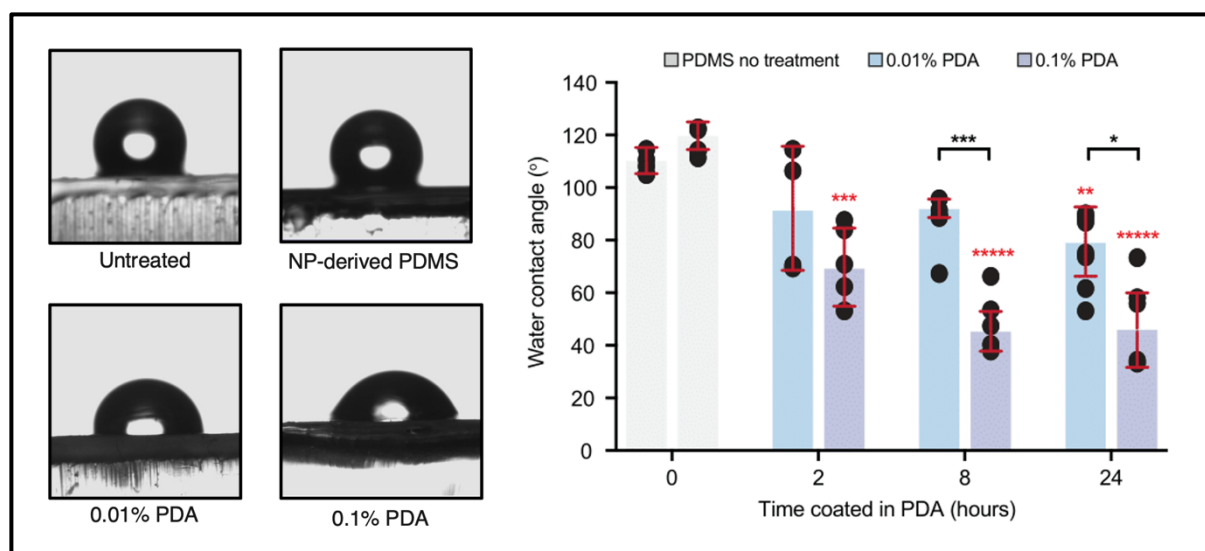


Figure 3.4: Polydopamine-coated PDMS significantly increases surface hydrophilicity.

(Left) Representative images of water droplets on PDMS surfaces are shown, with or without incubation, in 0.1% or 0.01% dopamine hydrochloride solution at pH 8.0. (Right) The mean contact angle of PDMS coated with 0.1% or 0.01% polydopamine is plotted for 0, 2, 8, and 24 hours of incubation in the coating solution. Error bars represent $\pm$ one standard deviation. Statistical analysis was performed using the Tukey HSD test: *p-value < 0.05, **p-value < 0.01, ***p-value < 0.001, ****p-value < 0.00001 ($n > 3$).

3.5 Discussion

PDMS microfluidic devices are extremely attractive for cell biological assays and research due to their high biocompatibility and ability to control the cell physiochemical microenvironment.¹⁸² Additionally, the reduction in reagent consumption, the scalability to multiplex (upwards of thousands of reactions per device), and the ability to create unique designs has greatly innovated cellular research, particularly in precision medicine and high-throughput drug screening.^{183, 193-196} Despite all these advantages, microfluidic systems have not been widely adopted by biology researchers, who are still using more traditional plate-based assays.¹⁹⁷ This is understandable, since plate-based methods have been optimized and standardized

and are incredibly simple to use and provide robust results. Several factors can be attributed to the gap between researchers and the use of microfluidic devices, including: (1) fabrication and (2) use of the device.⁸⁹ The fabrication process often requires specialized and expensive equipment, and the operation of microfluidic devices can be difficult and cumbersome for the user; both deterring the setup and use of microfluidic systems in biology research. To address the first obstacle, our work outlines an inexpensive and simple approach to rapidly generate optically clear PDMS microfluidic devices that can have extensive biological.

3.5.1 Commercial SLA 3D printers are useful tools in PDMS device fabrication

3D printing technologies have made great strides in biomedical and biomolecular research.^{133, 198, 199} Though 3D printing has been demonstrated to be a highly useful and emerging technology, it has not been widely adopted, since it still requires specialized equipment and user expertise. We intended to bridge this gap and provide a detailed guideline for the fabrication of PDMS devices using 3D printing technology. Our focus was to construct a simple and versatile method to generate PDMS microfluidic devices, from start to finish, with a product suitable for mammalian cell applications. We pushed the limits of the available FormLabs and AnyCubic 3D printers and showed that we could generate channel features in the <100 μm range, though larger feature sizes result in a more accurate final product. We were able to generate devices with small features (>25 μm channel height) that were still capable of fluid flow. This is, to the best of our knowledge, the lowest reported PDMS structure generated with 3D printing. Future developments in the field of 3D printing will maximize the resolution limit, while maintaining the accuracy of the final printed part.

3.5.2 Post-processing of 3D moulds is critical for optically clear devices

The potential applications for PDMS microfluidics are vast, spanning from diagnostic systems to sample testing and drug discovery.^{99, 178, 181, 200} To date, 3D printing has not yet produced PDMS microfluidic devices with the same level of resolution and clarity when compared to devices obtained from more traditional methods. This is largely due to the limitations of the printer, PDMS curing inhibition that may result from different types of photocurable commercial resins, and the smoothness of the resulting moulds that are generated by 3D printing. Our described post-processing steps can be applied in a wide range of laboratory settings, producing clear PDMS devices that approaches those created via photolithographic techniques.

The first critical component to our post-processing step is the heat treatment of the 3D print. Contingent on the type of resin used, most photocurable resins are comprised of acrylate monomers, crosslinkers, colouring agents/photo absorbers, plasticizers, and most importantly, the photoinitiator. Phosphine-oxide-based photoinitiator fragments can leach from the moulds and inhibit the PDMS catalyst.^{193, 201} In contrast, unreacted monomers strongly bind and attach PDMS to the 3D-printed surface. Both photoinitiators and unreacted monomers can remain even after the 3D printing process, and their combined actions result in PDMS curing inhibition. UV treatment maximizes the polymerization of the resin, and high-temperature treatment of the moulds allows any present photoinitiator to be vaporized, both processes reducing the presence of residual photoinitiator and resin monomer, enabling proper PDMS curing.¹⁰⁸ In line with these studies, we found that high heat treatment removes these photoinitiator fragments, enabling complete PDMS curation. Notably, moulds are susceptible to cracking and breaking at prolonged levels of dry heating at >100 °C, so we propose the use of shorter heat treatments or high heat treatment with

humidity (such as the autoclave), which does not visibly affect the structural integrity of the mould.

The second important post-processing step involves physical and chemical surface modifications to the 3D print to obtain a smooth surface. Several spray paint or lacquer coatings have previously been used to conceal the layer markings to 3D printed objects.^{124, 193} Similarly, chemical coats with SU-8 and fluorosilanes have been used to improve PDMS curing and clarity.¹³⁵ Furthermore, Parylene-C coatings have also been used to aid in PDMS release in addition to protecting master moulds, extending the number of cycles of moulding with various polymers. However, these methods require specialized pre-treatments and/or equipment.^{202, 203} We provide an alternative, low-cost, coating solution involving nail polish, which significantly increases the surface smoothness of the 3D printed moulds, resulting in extremely clear PDMS surfaces ($R_q < 33$ nm). In comparison, the surface roughness of Pyrex glass has been estimated to be $R_q = \sim 1$ nm.²⁰⁴ Although our fabrication method does not produce PDMS surfaces rivalling that of pure glass substrates, we outline a novel post-processing method that approaches the surface smoothness of glass and outperforms other reported methods that use 3D printing to generate PDMS devices.^{205, 206}

The final part of our post-processing outline involves the application of a coating to the PDMS surface to increase hydrophilicity. PDMS is a highly hydrophobic substrate. This is particularly problematic for cell biological applications, since adherent mammalian cells will not adhere to a hydrophobic surface.^{177, 207, 208} Additionally, air bubbles can readily form within a hydrophobic microfluidic device as hydrophilic media or solution are flowed through the device.²⁰⁹ Therefore, increasing

the hydrophilicity of the PDMS surface allows for biomedical applications and promotes the proper operation of the microfluidic device.

3.6 Conclusion

The use of photo-polymerization methods to generate master moulds and create microfluidic devices is an emerging field that is beginning to bring microfluidic capabilities and experimentation to researchers, even those that lack specialized microfluidic equipment. Since this field is still in its early stages, recent work, including ours, has been aimed to characterize these fabrication methods and provide guidelines to generate simple, efficient, and inexpensive devices.¹⁸⁵ As advancements in 3D printing technologies continue to push the resolution limit, we anticipate that our post-processing steps will remain a highly efficient and inexpensive method to produce highly transparent and functional PDMS devices.

4 Culturing HCEC cells in PDMS microfluidic devices fabricated using low-cost commercial 3D printers

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Author	Concept/Design	Data Acquisition	Analysis	Write up/Publication
Ramasamy	Y		Y	Y
Ho	Y	Y		
Phan	Y			Y
Jones	Y			Y

4.1 Introduction

In recent years, the adoption of lab-on-a-chip platforms for ocular research has gained significant attention.²¹⁰ This technology offers several benefits, including the capability to simulate tissue/organ functions, manage morphological and biochemical microenvironments, and enable real-time imaging.²¹⁰ By employing this strategy, a more physiologically appropriate model for studying ocular biological events and enhancing medication delivery systems can be developed.²¹¹ For instance, eye-on-a-chip and contact lens-on-a-chip models have been developed to partially simulate human ocular environments.^{42, 212} However, the conventional fabrication methods, such as soft lithography, which require a semi-clean environment and expensive equipment, pose the biggest challenge to the widespread use of this technology.⁵⁸

This chapter discusses using PDMS microfluidic devices manufactured with low-cost 3D printed mould to cultivate HCEC. The hydrophobic property of the PDMS surface poses a challenge to cell attachment and proliferation. PDMS was coated with PDA and Col to render its surface hydrophilic. PDA serves as an anchor for collagen and provides cell adhesion, while collagen promotes cell proliferation by simulating the natural extracellular matrix environment.¹⁵⁷ PDA/Col coated PDMS devices have been utilized for culturing mesenchymal stem cells and fibroblast cells.^{157, 213} In our study, this PDA/Col combination was employed to modify the surface of our PDMS devices fabricated using the 3D printed mould to incorporate HCEC.

4.2 Materials and methods

4.2.1 Fabrication and post-processing of PDMS microfluidic devices

The PDMS (Sylgard 184, Dow Corning, USA) elastomer and curing agent were combined in a ratio of 10:1 to make a homogenous mixture, which was then

centrifuged at 500 g for 10 minutes to eliminate air bubbles. The resulting PDMS mixture was cast into the post-processed 3D-printed master moulds and cured at 75 °C for 1.5 hours. The resulting PDMS devices were gently released from the moulds and sterilized by autoclaving. Then, to enhance the hydrophilicity of PDMS, they were dip-coated in two different concentrations of PDA (0.001%, 0.01%) solution for 24 hours. Subsequently, the PDMS devices were dip-coated in a 20 µg/ml collagen (Sports Research, CA, USA) solution. Both PDA and collagen solutions were prepared by dissolving them in PBS at pH – 8.5.

4.2.2 Cell culture and cell seeding in PDMS device

Immortalized HCEC were grown in standard polystyrene tissue culture plates supplied with DMEM/F12 medium with 1% fetal bovine serum (ThermoFisher Scientific 16000044, Waltham, MA, USA) and 1% penicillin-streptomycin (Fischer Scientific 15140122, ON, Canada). The cultures were maintained in an incubator at 37 °C and 5% CO₂ until they reached 80% confluence. When the HCEC attained a confluent state, standard EDTA/Trypsin (Sigma-Aldrich T3924-500ML, St. Louis, MO, USA) solution was utilized to separate them from tissue culture plates. In a standard 96-well plate, cell seeding is typically carried out at a density of 10,000 cells per well, where each well has a surface area of approximately 0.32 cm². However, when using PDMS (Polydimethylsiloxane) devices, the well surfaces are only 60% of that found in tissue culture plates. To maintain an equal surface area for cell growth, approximately 6,000 cells were injected into the sterilized PDMS devices using a syringe, while varying the treatment conditions. These cells were allowed to grow in DMEM/F12 media at 37 °C in a high-humidity environment for 36 hours.

4.2.3 Cell viability assay

Following the incubation period, the HCECs were visually assessed for their morphology, including their shape, size, adherence, and any potential abnormalities, to gain insights into their overall health using bright field microscopy. Next, the cell viability assay was performed using Alamar blue reagent (ThermoFisher Scientific DAL1025, Waltham, MA, USA) to validate the metabolic activity and viability of HCEC colorimetrically. The viability assay works on the principle that the Alamar blue reagent penetrates the cell membrane of viable cells and gets oxidized, resulting in a colour change from blue to pink. HCEC were grown on PDMS devices subjected to varying surface treatments including no treatment, only Col, only PDA (0.001%), 0.001% PDA/Col, 0.01% PDA/Col and compared with HCEC grown on standard tissue culture plate (Corning® 96 Well TC-Treated Microplates, Millipore Sigma CLS3595-1EA, Oakville, ON, Canada). To each microfluidic device, 10 µL of Alamar blue reagent was added into the wells (~1:10 dilution), and the cells were incubated for 4 hours. After this incubation period, 100 µl of the sample was extracted from the microfluidic device. Subsequently, a 100 µl sample was collected from the cells grown in standard tissue culture plates, which is the control. The fluorescence of the extracted sample was measured using the Imaging Multimode Plate Reader (Cytation 5, Agilent BioTek Instruments, Winooski, VT, USA) with excitation and emission wavelengths of 540 nm and 590 nm, respectively. The fluorescence intensity, which corresponds directly with the number of viable cells, was recorded.

4.2.4 Statistical analysis

The statistical analysis and graphs were generated using GraphPad Prism 8 software (GraphPad, version 8.2.1, La Jolla, CA, USA). Six samples were used for the cell viability tests, and the results are presented as the mean and standard deviation.

Using the Shapiro-Wilk test, the normality of each group was evaluated separately. A one-way ANOVA was used to determine if there was a statistically significant difference in the proportion of viable cells between treatment groups. Tukey's post-hoc test was applied to pairwise cell viability comparisons, revealing substantial differences. For all analyses, $p < 0.05$ was used as the significant level.

4.3 Results

4.3.1 PDA/Col coated PDMS devices exhibit increased cell viability compared to untreated PDMS devices

The integration of HCEC into surface modified PDMS was investigated. As HCECs play a crucial role in ocular drug delivery and eye health maintenance, they were chosen for this study. HCEC proliferation and adhesion to surface modified PDMS devices were visually assessed utilizing brightfield microscopy images (Figure 4.1A). This indicated that the surface modification technique improved cell adhesion.

Next, we tried to investigate the viability of HCEC with increasing PDA concentrations and PDA/Col combinations (Figure 4.1B). With increasing PDA concentration, we observed a significant increase in cell viability ($p < 0.001$). This considerable difference indicated that PDA was essential for improving the cell compatibility of PDMS devices. Interestingly, there was no significant change in cell viability between collagen-treated and untreated PDMS devices. Moreover, the cell viability of PDA/Col-treated PDMS devices increased from 13.5% to 80.2% compared to untreated PDMS devices ($p < 0.001$). Though cell viability increased to 80% for PDA/Col treated PDMS devices, the cell viability percentage was significantly lower when compared to standard tissue culture plates ($p < 0.0001$). This result underlines

the need for continued investigation and optimization to bridge the gap and maximize the potential of PDMS devices in cell applications.

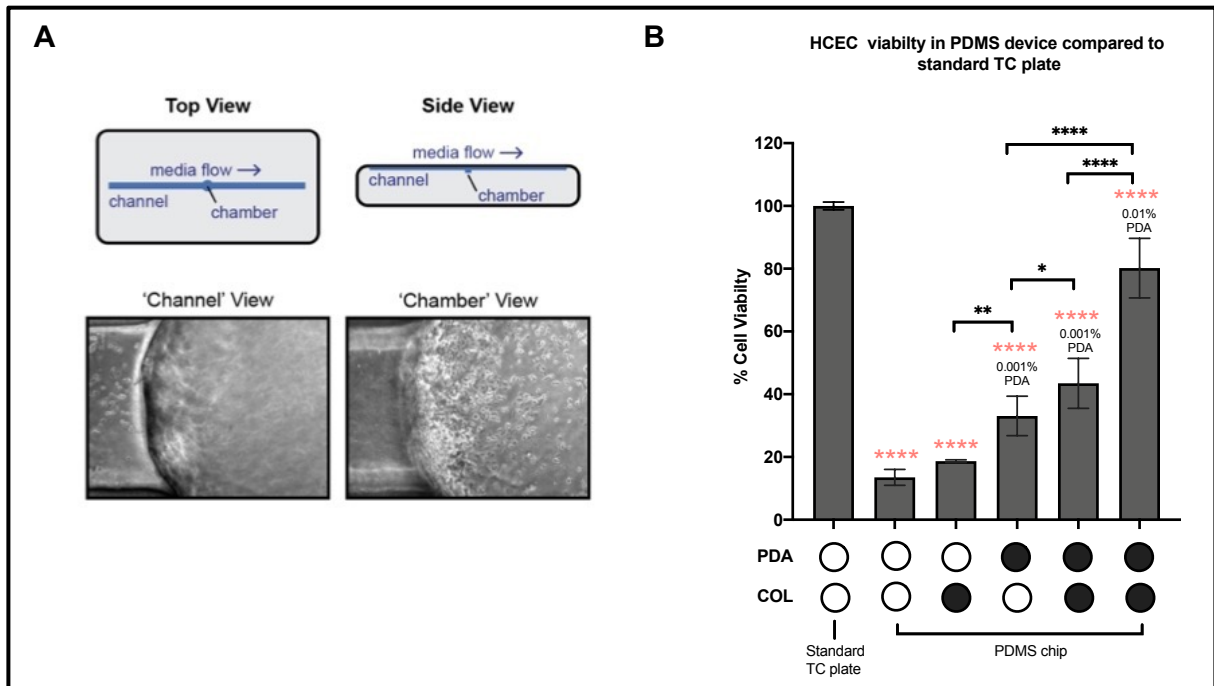


Figure 4.1: HCEC viability in PDMS device compared with standard tissue culture plate.

A) Brightfield microscopy image of HCEC cells grown in PDMS chip B) Cell viability % of HCEC grown in PDMS device coated with only PDA, only Col, 0.001% PDA/Col, 0.01% PDA/Col compared with standard tissue culture plate. Statistical analysis was performed using the Tukey HSD test. *p-value < 0.05, **p-value < 0.01, **p-value < 0.0001 (n=6).**

4.4 Discussion

Eye-on-a-chip technology has gained immense popularity in recent years due to its ability to simulate physiological ocular conditions, generating more precise findings than conventional 2D cell culture models.^{64, 214} However, the adaptability of this technology is limited due to the high cost and technical expertise required in the fabrication process.⁵⁸ This study aimed to develop an HCEC-PDMS device using low-cost commercial 3D printers, making this technology accessible to all.

The hydrophobic property of PDMS, which hinders cell growth, is one of the most significant obstacles associated with its use. To circumvent this limitation, we deployed PDA and Col, two biopolymers recognized for their cell proliferation and adhesion capabilities. PDA, well-known for its adhesive qualities, rendered the surface hydrophilic, facilitating initial cell attachment. Additionally, collagen, a vital extracellular matrix component, stimulated cell proliferation.^{151, 157, 215}

PDA/Col-treated PDMS devices revealed increased HCEC viability compared to untreated PDMS devices, yet still lagged behind standard tissue culture plates. Our observation was consistent with previous research that reported low cell viability (<40%) for untreated PDMS devices. Surprisingly, these investigations also revealed that when cells were cultured over an extended duration of 72 hours, their viability might increase by up to 120 %.²¹³ Comparatively, our study, conducted within 36 hours, achieved 80% cell viability. This study demonstrates that with further optimization and extended cell culture durations, our HCEC-PDMS device could potentially maximize cell viability.

HCECs are adherent cell types, and they must be adhered to the PDMS surface in order to maintain their morphology and function. This attachment can be mediated by cell adhesion molecules like integrins, which not only facilitate adhesion but also regulate cell signalling and activate the metabolic activity of the cells. However, HCEC not adhered to the PDMS surface can exhibit reduced metabolic activity.²¹⁶ Alamar Blue assay cannot directly measure cell adhesion and can only assess cell viability and metabolic activity. The assay works by measuring the reduction of resazurin, a non-toxic, cell-permeable dye, to resorufin by mitochondrial enzymes in living cells. The amount of resorufin produced is proportional to the number of viable cells in the sample.²¹⁷ While the Alamar Blue assay can indirectly provide information about cell

adhesion by measuring the metabolic activity of cells that adhere to a substrate, it does not directly measure the number of cells attached to the PDMS surface. Other assays, such as crystal violet staining and the quartz crystal microbalance, fluorescence-based counting are better suited for directly measuring cell adhesion.²¹⁶

4.5 Conclusion

In this study, we successfully developed an HCEC-PDMS device using low-cost 3D printers available on the market. To overcome the limitation of PDMS surface hydrophobicity, PDA and Col were successfully used to modify the surface of PDMS. Our research demonstrates that this technology has the capability to create better realistic models for ocular research, with promising improvements in cell viability.

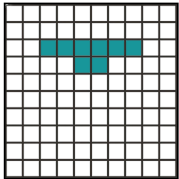
5 General discussion and future direction

Cytotoxicity screening is a crucial step in assessing the toxicity of newly developed therapeutics in preclinical studies. It serves as a link between preclinical investigations and clinical trials.²¹⁸ Nonetheless, the vast majority of medications fail in clinical trials due to a lack of efficacy and unforeseen toxicity issues.^{169, 219} For instance, in phase II clinical trials, greater than 50% of drug failure is attributed to toxicity.²¹⁹ The primary reason for this is the lack of sophisticated *in-vitro* ocular models that could better validate the safety of therapeutics.¹⁶⁹ In recent years, researchers have attempted to create an eye-on-a-chip model utilizing PDMS chips.¹² The major hindrance in accessing the PDMS chips is the lack of a simple, cost- and time-efficient, scalable fabrication method.⁵⁸

5.1 Experimental summary and discussion

As described in Chapter 3, 3D printing is an emerging fabrication technique that offers an alternate method for resolving the challenges associated with the fabrication of microfluidic chips. This chapter described the fabrication of PDMS chips from a 3D printed mould using Formlabs and AnyCubic 3D printers (Table 5.1). Three simple and cost-effective post-processing stages were described to generate optically transparent and smooth PDMS chips: heating, sanding, and coating with nail polish. The heating of 3D printed moulds was vital in ensuring complete PDMS curing by eliminating any residual uncured resin on the mould surface, as validated through Brightfield microscopy. Subsequently, the combination of sanding and nail polish coating produced smooth and optically transparent PDMS chips. Furthermore, the hydrophobic PDMS surface was rendered hydrophilic by dip coating of PDA.

Table 5.1: Comparative analysis of 3D printers and their resolution

3D printer	Formlabs Form 3B ⁺	AnyCubic D2 DLP
Resin used	Clear resin	Black resin
Technology	Low force stereolithography	Digital Light Processing
Light source		
Cost	\$ 8000	\$ 650
Printing time/chip	150 minutes	45 minutes
XY resolution (manufacturer description)	25 μm	51 μm
XY resolution observed	The printed widths varied from the intended design values, having deviations of 300% to 25% for actual designed widths ranging from 25 to 500 μm , respectively.	The measured resolution of the printed objects precisely corresponded to the intended resolution, indicating accurate replication without any discernible deviations.
Angle deviation of channels	Channel dimensions >200 μm had less than 10° deviation	Channel dimensions >200 μm had less than 10° deviation

During the investigation, it was discovered that the printed channels exhibited a variation of 100 μm in comparison to their intended dimensions for the Formlabs 3D printer. Additionally, for channel dimensions below 200 μm , both Formlabs and AnyCubic 3D printers exhibited an angular deviation of up to 20°. While designing the chip, these factors must be considered. Intriguingly, our findings corroborated prior studies indicating that the resolution of the least expensive 3D printers, such as AnyCubic, was comparable to that of the medium-priced 3D printers, such as Formlabs.²²⁰

The development of microfluidic chips for ocular cell applications began with the direct 3D printing of chips utilizing Formlabs 3D printer with clear resin. In our preliminary findings, we discovered that the channels were blocked by resin below 800 μm . This was in line with the previous findings stating only a 65% accuracy for channels with dimensions of 500 μm and complete blockage of channels below 250 μm .²²¹

In a subsequent design iteration, a reverse mould was 3D printed using Formlabs and AnyCubic 3D printers. When PDMS was cast in the 3D printed moulds from both 3D printers, PDMS curing inhibition was observed, a common issue associated with photopolymer-based resin 3D printers.¹⁰⁸ UV curing for prolonged periods (60 °C, 2 hours) resulted in partially cured PDMS with minimal adhesion in the case of Formlabs. PDMS curing was strongly inhibited when the identical curing process was applied to 3D-printed moulds from AnyCubic 3D printers. This disparity in PDMS curing inhibition can be attributed to the proprietary resin formulations, as UV penetration depth differs amongst resin types.¹⁰⁸ This led to the inclusion of a heating step (100 °C, 2 hours) along with UV curing. To achieve completely cured PDMS chips, a minimum of 2 hours of heating was essential. When heated for more

than four hours, it resulted in the moulds breaking. UV curing of 60 °C for 30 minutes and heating for 2 hours resulted in completely cured PDMS for 3D printed moulds from both 3D printers. Table 5.2 summarizes the treatment conditions required to produce fully cured PDMS chips from both Formlabs and AnyCubic 3D printers.

Table 5.2: Treatment conditions for 3D printed moulds from Formlabs and AnyCubic 3D printers

	PDMS curing inhibition	Complete PDMS curing
Formlabs & AnyCubic	Less UV curing, no/less heating	Less UV curing, more heating
	Long UV curing, no/less heating	Long UV curing, more heating

Table 5.3: Comparison of surface treatments for 3D printed moulds

Surface Treatment	Ease of application	PDMS removal from the mould	Optical clarity
Sanding (1500 to 15000 grit)	Moderate	Easy	Improved
Polyvinyl alcohol (PVA) coating	Easy	Moderate	Opaque
Silicone mould release spray	Easy	Easy	No improvement
Medical-grade adhesive tape	Moderate	Easy	Improved
Polyurethane spray coating	Easy	Difficult (PDMS curing inhibition)	No improvement
Nail polish coating	Easy	Easy	Improved
Sanding + Nail polish coating	Moderate	Easy	Best of all

The subsequent challenge was generating optical transparent PDMS chips from 3D printed moulds. The PDMS obtained from Formlabs and AnyCubic moulds were translucent, rendering it unsuitable for cell applications requiring microscopic imaging. The translucency of the PDMS was attributed to the surface roughness of the 3D printed moulds.²⁰⁵ Our goal was to design a low-cost, straightforward surface treatment for 3D printed moulds to obtain smooth, optically transparent PDMS chips. As a result of sanding the surface of the 3D printed mould, the optical clarity (50 to 60 %) was improved, but not enough to distinguish cells under a microscope. Then, we experimented with a range of coatings, including PVA, mould release spray, medical-grade adhesive tape, polyurethane spray, and nail polish coating (Table 5.3).

The ultimate challenge included transforming the hydrophobic surface of PDMS, as this inhibited cell proliferation and adhesion. Initially, GelMA was pumped using a syringe pump and cured with a portable UV curing device. As the surface of PDMS was not plasma-activated, GelMA served as a discrete, non-interacting layer, complementing the previous studies.²⁰¹ Moreover, pumping GelMA into each channel is not efficient or scalable. In the final iteration, PDA was applied as a biocompatible coating on PDMS surfaces. PDA coating does not require surface plasma activation, and it can bind to PDMS via its functional groups, hence enhancing cell contact and enabling conjugation with other biopolymers.^{151, 215} Instead of a syringe pump, simple and scalable dip coating was utilized to apply PDA coating on PDMS substrates. Water contact angle measurements validated a decrease in PDMS surface hydrophobicity.

The next crucial factor was chip design and bonding methodology. It began with a simple three-channel layout measuring 65 mm in length, 1 mm in height, and 1 mm in width. It was challenging to incorporate cells across the entire channel. Cell chambers (cylindrical wells) were incorporated in the following layout to accommodate

cells. Initially, plasma bonding techniques were employed for bonding. The plasma bonding of PDMS resulted in robust, permanent bonding. However, not all labs have access to this expensive equipment. Instead, PDMS bonding with medical-grade adhesive tape was employed, ensuring no fluid leakage up to 100 $\mu\text{L}/\text{min}$ flow rate. Consider the criteria listed in Table 5.4 while designing chip moulds.

Table 5.4: Chip design considerations

Chip design	Description
Channel length	Incorporate smaller channel lengths to minimize pressure and avoid leakage during fluid pumping for extended periods.
Channel dimension	Design channels with equivalent width and height to ensure proper printing and avoid issues with print quality.
Print orientation	Vertical (90°) - Avoid due to longer print time and potential breakage Horizontal (0°) - Recommended as shorter print time (Note: Ensure no cupping effect) Slanting ($15\text{-}45^\circ$) - Highly recommended for better print quality and reduced peel forces

Chapter 4 discussed the integration of HCEC into the PDA/Col surface-modified PDMS that was generated using a post-processed 3D-printed mould. HCEC demonstrated enhanced viability on Col/PDA-treated PDMS chips compared to untreated PDMS chips, yet this difference was not statistically significant compared to standard tissue culture plates. In the present study, a cell viability experiment was performed for 36 hours. Future studies will attempt to perform cell viability experiments for an extended timeframe. Furthermore, future research will investigate the survival of cells at varying flow rates and the influence of shear stresses on HCEC. Moreover, the study could be expanded to include other ocular cell types, creating co-culture systems resulting in sophisticated *in-vitro* eye models.

PDA is a versatile and biocompatible polymer with a unique combination of catechol and amine functional groups, which enhance its surface adhesion properties.^{222, 223} The one-step coating process of PDA makes it a very attractive biomaterial coating. Dopamine-hydrochloride undergoes an oxidation reaction under alkaline conditions, forming PDA by a simple dip coating or spraying process on any surface, including organic and inorganic surfaces. The thickness of the PDA coating can be controlled by parameters including dopamine concentration, temperature, coating time, and pH of the coating solution.^{97, 224} It has been reported that coating thickness is linearly proportional to the coating time and PDA concentration. With increasing coating thickness, the roughness of the PDA-coated surface increases. However, there is no proper correlation between the effect of coating thickness on cell growth, and it mostly depends on the cell type. This is evident from the research that 24 hours of PDA coating promoted the adhesion of chondrocyte cells, while 5 minutes of PDA coating time was sufficient for the adhesion of myoblast cells.^{222, 224}

PDA has been shown to promote cell adhesion, expansion, and proliferation *in-vitro* for various cell types, including human umbilical vein endothelial cells,²²⁵ human mesenchymal stem cells, myoblasts,²²⁶ and chondrocytes.²²⁷ Ku et al. demonstrated for the first time that surfaces such as polyethylene, polytetrafluoroethylene, silicone rubber, and PDMS, when coated with polydopamine were non-toxic to mammalian cell lines MC3T3-E1 and PC12, used for bone and nerve regeneration respectively. The cell adhesion and viability are dramatically increased several folds up to a maximum of 5.46 compared to unmodified bare surfaces.²²⁸ Niedo et al. studied the cytotoxicity of PDA nanoparticles on different types of human carcinomas, such as breast, colon, liver, and lung. The findings indicated that as the size of the PDA nanoparticles decreased, their antiproliferative activity (ability to slow down the growth of cancer cells) became more pronounced. Additionally, the lowest tested concentration exhibited a tumor-selective effect.²²⁹ Baidukova et al. showed that PDA had no cytotoxicity on A549 lung cancer cells up to a maximum concentration of 2.5 mg/mL. However, PDA above 0.2 mg/mL exhibited cytotoxic effects on neural cells, leading to decreased metabolic activity and cell death.²³⁰ These studies have mostly incorporated PDA as an encapsulation for drug delivery and coating in biomedical implants. Most studies have shown that PDA can be non-cytotoxic and used with a wide range of cell types at lower concentrations. However, caution should be exercised when using higher concentrations of PDA, especially with neuronal cell types.

5.2 Addressing limitations and suggestions

The proposed fabrication methodology provides an efficient and economical method to generate optically clear and smooth PDMS chips utilizing affordable 3D printers. Nevertheless, there are a few constraints that need to be addressed.

Firstly, the sanding and applying a nail polish coating to attain a smooth surface on 3D printed moulds inadvertently led to changes in the channel dimensions. To circumvent this limitation, advancements in 3D printing technology are essential. For instance, considering the Formlabs SLA printer, the XY resolution is attributed to the step size of the Light Processing Unit, which is 25 μm (how precise the laser can be moved), and the laser spot size is 80 μm (diameter of the laser). Advancements in the laser source and light processing unit, combined with a more flexible resin tank (which reduces peel forces), could result in high-resolution 3D prints with a smoother finish, eliminating the need for post-processing.

Secondly, the heating step, which is performed to vaporize the uncured resin monomers (acrylate-based) on the mould surface, can result in mould deformations, affecting the reusability of mould. By developing novel resin formulations that do not affect the PDMS curing process, the heating process can be eliminated, increasing the reusability of the mould.

Thirdly, PDMS is commonly used for cell applications, but its hydrophobic nature poses challenges for cell adhesion. The 3D printed mould can be used to cast other hydrogel biopolymers. This can eliminate the need for surface modification strategies, as hydrogels possess inherent properties to promote cell adhesion and proliferation. However, it is also necessary to consider the degradation factor of hydrogels and choose biopolymers in combination.

There are several other challenges in the fabrication of PDMS devices that need to be addressed in order to optimize the performance and functionality of these devices for cell applications. These challenges include leakage of fluid, air bubble formation in channels, and flow rate controllability.

Fluid leakage poses a significant challenge in the utilization of microfluidic chips. Due to its small volume, even a minute leakage can affect the experimental study. According to the Hagen-Poiseuille equation, the pressure drop is inversely proportional to the third power of the channel height for rectangular channels.²³¹ This indicated that when the channel is very narrow, high pressure is created inside the channels which is prone to leakage.²³¹ The first step in preventing leakage is to ensure tight bonding of the PDMS device with a glass slide. In our study, PDMS devices were bonded to glass slides via a medical-grade adhesive tape. Though this method could withstand pressure up to 891 Pa, its bonding strength is lower when compared with plasma bonding⁹⁴ and prone to leakage if not bonded properly. The integrity of bonding can be visually identified through the flow of dyed solutions in channels using a syringe pump for prolonged hours.²³¹ Additionally, this step can aid in determining the need for change in channel design if required. Furthermore, the surface roughness of PDMS can affect the bonding of PDMS, which can lead to fluid leakage.²³² Hence, having a smooth PDMS surface could prevent potential fluid leakage. In our study, the 3D printed mould was treated with sanding and nail polish coating to obtain a smooth PDMS surface.

Syringe pumps are widely used in microfluidics to pump fluids as they provide a more stable flow, simple and easy to use. However, the syringe pump enables the accurate flow of fluids; continuous pumping of fluid can result in fluctuation, which adds to the build-up of pressure within the channels. This fluctuation can result in channel deformation and non-uniform flow rate within channels. The former might result in cell death due to increased cell stress, while the latter might produce inaccurate cell viability results as the nutrient supply can be uneven.^{233, 234} These challenges can be overcome by modifying channel design, including separate channels for cell injection

and vascular network-shaped channels for media flow that can provide a uniform supply of nutrients.²³⁴ Next, a smaller syringe diameter can improve flow stability at low flow rates as the minimal volume dispensed is directly proportional to the syringe diameter used. Additionally, a feedback loop can be introduced to the syringe pump, which can control the pressure induced by the pump and regulate the fluid flow in a stable and precise manner.²³³

PDMS being a gas-permeable and hydrophobic material, one of the significant challenges in employing it in cell studies is the formation of air bubbles. Factors such as changes in temperature, pressure, dissolved gasses in fluid, and hydrophobic surface can contribute to the formation of air bubbles.²³⁵ Air bubble formation can induce flow instability by blocking the channel. For cell applications, they can cause stress on cells, resulting in cell death or alteration in their morphology, which can potentially affect the experimental study.²⁰⁹ By converting the surface of PDMS hydrophilic, the air bubble formation can be prevented. In our study, the PDMS was made hydrophilic by coating with PDA. Furthermore, the air bubble formation can be prevented by ensuring the microfluidic setup is placed in a chamber or environment with constant temperature and pressure. A series of hemispherical wells can be created in the PDMS devices acting as a bubble traper.²³⁶ Additionally, ensure the PDMS devices are tightly sealed and the connection between the tubings and syringes is intact to prevent the formation of air bubbles.

5.3 Future work

In addition to the work presented, our research group attempted to create a contact lens chip using the 3D-printed mould. In laboratory investigations, drug release studies from contact lens devices are performed in simple vials containing a

predetermined amount of tear fluid.²³⁷ These vial models lack certain features of the human ocular system, such as tear flow and low tear volume.²³⁷

We designed a contact lens chip consisting of a top and bottom component, simulating a low tear flow environment using a syringe pump. The upper portion comprised an inlet, outlet, and channels for fluid flow. The lower section contained a dome-shaped mount to mount a contact lens. The top and bottom portions can be fused using double-sided adhesive tape. This contact lens platform is envisioned to be connected to the HCEC chip (Figure 5.1), evaluating the toxicity of therapeutics released from contact lens materials.

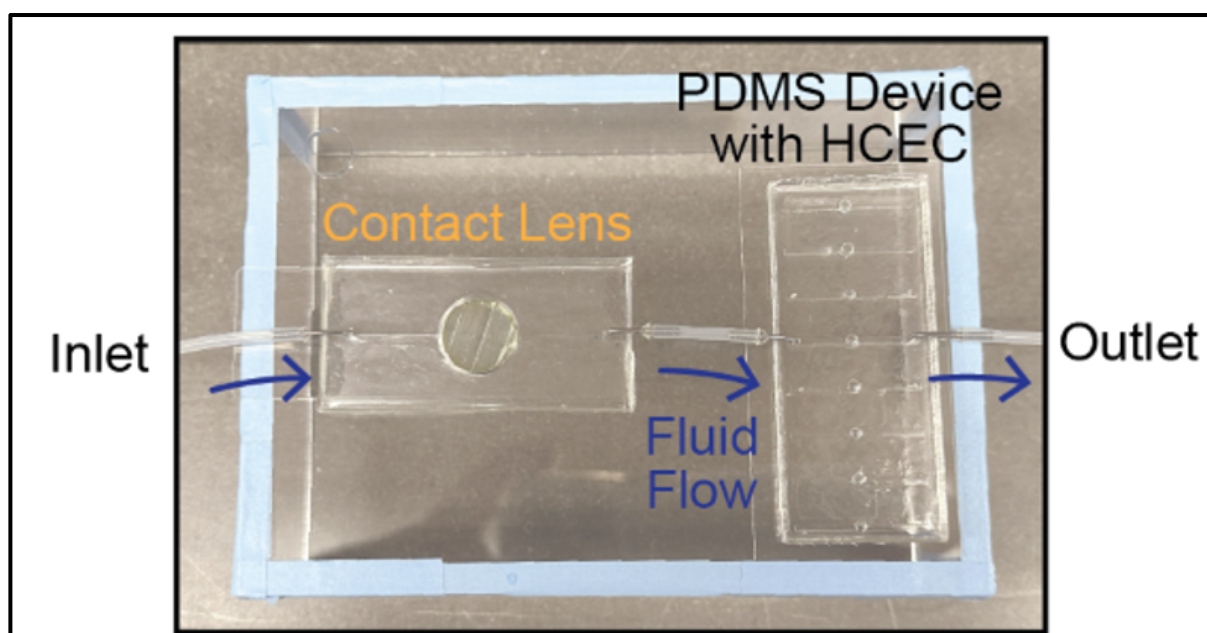


Figure 5.1: Integration of a contact lens chip module with the HCEC chip module. (Courtesy: Dr. Brandon Ho)

5.4 Conclusion

This thesis aimed to establish simple post-processing techniques for fabricating PDMS devices using low-cost, commercially available 3D printers that could be used for ocular cell applications. We fabricated optically clear and smooth PDMS devices by incorporating post-processing steps like heating, sanding, and nail polish coating.

However, sanding and nail polish coating steps resulted in variations in the channel dimensions of moulds. Innovations in 3D printing technology and the creation of novel resin formulations can be crucial in overcoming this constraint. This work paves the way for further research and development in fabricating cost-effective, customizable PDMS devices for ocular cell applications.

6 References

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