3D Cyclic Olefin Copolymer (COC) Microfluidic Chip Fabrication Using Hot Embossing Method for Cell Culture Platform

by

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Bachelor of Science in Mechanical Engineering
Massachusetts Institute of Technology, 2008

Submitted to the Department of Mechanical Engineering in partial fulfillment of the requirements for the degree of Master of Science in Mechanical Engineering at the MASSACHUSETTS INSTITUTE OF TECHNOLOGY

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Abstract

A microfluidic system has been developed for studying the factors inducing different responses of cells in vascular system using a three-dimensional microenvironment. The devices have been transferred from PDMS to a platform in cyclic olefin copolymer (COC) which has advantages in terms of hydrophobicity, production by the more commercially-viable hot embossing technique, and amenability to surface treatments. Here the fabrication process is described and the new systems are characterized. Surface wettability, bond strength between the system body and a covering plastic film, and cell viability data are presented and compared to systems fabricated in PDMS.

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All information in this thesis is protected by invention disclosure submitted in Oct. 2009. The case number for the US patent application is 61/248,603, and docket number is DPL-134PR. The same case was assigned a different case numbers at Draper Laboratory and at MIT: CSDL 2057, and MIT 12994.
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Chapter 1
Introduction

Numerous studies have examined the effects of various factors, both biochemical and biophysical, on cell morphology and function. Most of these have been conducted in standard cell culture systems consisting of culture wells, simple flow chambers or on stretchable substrates in which typically one, or a small number of factors can be controlled and studied. Recently, microfluidic systems have been reported that provide a wide range of capabilities including establishment and control of biochemical or thermal gradients, improved imaging, and the culture of multiple communicating cell types in a single in vitro device [1-3]. Much of our recent work has focused on vascular angiogenesis and the design of microfluidic systems optimized for its control [4, 5] and investigation [6, 7]. In these systems, cells are seeded via one flow channel on the surface of a scaffold material and then subjected to controlled concentration of growth factors, surface shear, or trans-endothelial pressure, inducing the formation of vascular sprouts that extend across the scaffold to a second flow channel. Our previous work has demonstrated the capabilities of our microfluidic platform to control biochemical and biophysical factors that influence angiogenic sprouting and cell migration that is developed and
fabricated in polydimethylsiloxane (PDMS) [4-6, 8]. Its capabilities as a device for inducing as well as quantifying angiogenesis have been amply demonstrated in variety of settings.

Material selection and fabrication methods can largely dictate performance, applicability, and manufacturability of a microfluidic device. Although successful microfluidic devices have leveraged convenience and utility of cast PDMS, it has several limitations. Devices made of PDMS are not optimal for studies involving pharmaceuticals because of porous hydrophobic and adhesive surfaces of PDMS, which may lead to absorption of small molecules such as hormones and adsorption of proteins [9-12]. The concentration of specific molecule cannot be determined exactly if the device material absorbs small molecules, and protein adsorption on devices can result in different signaling or cell differentiation. In addition, PDMS is not a viable option for mass production and automation. The soft lithography method of fabricating PDMS devices is rate-limiting and not suitable for high-throughput manufacturing, and its low modulus may allow change of device dimensions during operation.

Thermoplastics polymers have several advantages including the low material cost, availability of range of material properties tailored for specific need, and amenability for high-throughput fabrication [13-17]. Among many thermo plastic materials, cyclic olefin copolymer material has good optical properties, and good chemical properties such as low water resistance [18]. Access to phase and fluorescent imaging are one of big advantages of using microfluidic device for cell culture, and therefore good optical property is an essential factor in determining the material, and the COC material exhibits high transparency and optical clarity as well as low autofluorescence, and the strong chemical resistance and low water absorption are also advantages in selecting COC [14]. We used hot embossing process for replicating the design to COC material with epoxy master, and thermal bonding to laminate thin film to embossed pattern.
Epoxy masters are widely used in hot embossing since they are durable and inexpensive [19, 20]. Hot embossing process is high throughput in nature and will facilitate the transition to higher volume manufacturing for more clinically relevant sample sizes, and COC material will allow control over surface treatment which is crucial for the cell function while maintaining optical access.

Determining the contribution of each biomechanical and biochemical factors to the cells is difficult unless there is in vitro experimental platform. In this paper, we have describe new methods to fabricate microfluidic platforms for cell cultures using thermo plastic COC material and hot embossing process. We also investigate the quality of the replication process, characterize the hydrophilicity of the material, and demonstrate cell viability in COC devices. Since the technology described enables careful control of parameters that can replicate many in vivo conditions, we anticipate that adapting this technology will have significant implications in elucidating the mechanisms of cellular interactions. Moreover, the potential of a microfluidic system that can be manufactured inexpensively and in a high-throughput manner is enormous. The system could be used by the pharmaceutical industry for drug screening in a clinical settings to screen for optimal patient-specific drug protocols, and in research settings as a device for \textit{in vitro} scientific study of cells in a physiologically relevant context.
Chapter 2

Background

2.1 Microfluidics for cell culture

Cell migration is essential for both physiologically and pathologically for processes such as angiogenesis, cancer metastasis, wound healing and inflammation. There are many biophysical and biochemical factors identified to be contributing to cell migration and proliferation [21]. However, there have been limitations in understanding crosstalk between these components such as growth factors, interstitial flow or extra cellular matrix stiffness, despite comprehensive understanding of these components independently [22-25]. Therefore, there is an apparent need for an in vitro system to assist overall understanding how these factors act together in processes mentioned earlier. While the typical cell culture assays were unable to incorporate multiple factors the microfluidic cell culture platform that is developed can be applied to make the in vitro assay more physiological and adaptable to various applications.

2.1.1 Previous methods

Many assays for cell migration study have been used in the past. However, most of those assays, including wound assay and TEFOLON® fence assay, enable cell migration studies only in 2D,
which are not physiological. The difference that cells experience in 2D versus 3D system may cause cells to exhibit different morphogenesis. Furthermore, cells may totally act differently depending on 2D or 3D environment such that some cells will not exhibit metastatic behavior in 2D while the same cells will be metastatic when placed in 3D environment [26-29]. While the Boyden chamber has been claimed to most closely resemble 3D environment, it is not suitable for real time quantifiable cell migration experiments.

![Schematic of previous in vitro model](image)

Figure 2-1: Schematic of previous in vitro model [30]

Former assays did not permit the study of physiological factors such as a fluid-matrix interface and fluid flow experienced by endothelial cells in vivo. Furthermore, differentiating the chemokinetic behavior, which is increased motility in the presence of a particular biochemical factor, and chemotactic behavior, which is cell migration towards chemoattractant, were difficult using former methods. More difficulties included maintaining a controlled gradient, obtaining quantitative data from experiments and having optical resolution in real time.
2.1.2 Microfluidics in PDMS

The microfluidic system has been developed in polydimethylsiloxane (PDMS) using a soft lithography method. The PDMS microfluidic device enabled mimicking of the physiological environment with a 3D gel region for cells to attach and proliferate and migrate. Its capabilities of inducing and quantifying angiogenesis have also demonstrated to show that the device can indeed fulfill the objective of studying capillary morphogenesis [4-6]. One example of PDMS microfluidic device developed for endothelial cell and hepatocyte co-culture to study transport-mediated angiogenesis is shown in Figure 2-2. This was done by seeding endothelial monolayer cells in the bottom of the microfluidic channel and introducing tumor cells in different channel for the tumor cells to migrate through when chemoattractants are added to the system. The two channels with normal and tumor cells are separated by gel-like three-dimensional scaffold to mimic the tissue of vascular system. The Using such methods, the potential of the microfluidic platform to control biochemical and biophysical factors that influence angiogenic sprouting and cell migration have been demonstrated.
2.2 Limitations in previous system

The current experimental device is made from PDMS, which poses several problems. The hydrophobic and porous nature of PDMS is not appropriate for pharmaceutical studies. Because of hydrophobic surfaces of PDMS, proteins, which are also hydrophobic become easily attracted to PDMS. Adsorption of proteins to PDMS surfaces may lead to different differentiation of cell as well as triggering different signaling pathways [11, 12]. Figure 2. below shows site-specific

---

Figure 2-2: Schematic of microfluidic device and experimental images for endothelial cell and hepatocyte co-culture to study transport-mediated angiogenesis [5]
phosphorylation of focal adhesion kinase (FAK) depending on surface chemistries. As cross-linked polymeric network, PDMS is porous in nature, and when combined with hydrophobic surface properties, PDMS may absorb small molecules such as estrogen [10]. This may limit function of the device as various levels of topography, chemistry, surface energy, and hydrophobicity restricted to specific areas of the device may enable new functionalities. Uniform surface treatments would not allow these new functionalities. Specifically 3D gel retention and cell adhesion cannot be modulated with a uniform surface treatment, as well as control of protein adsorption and activity.

![Figure 2-3](image)

Figure 2-3: Site-specific phosphorylation of focal adhesion kinase (FAK) depending on surface chemistries [12]

Furthermore, PDMS is not a viable option for mass production because of difficulties for automating the process for making devices in PDMS. The soft lithography method of fabricating PDMS devices is rate-limiting and not suitable for high-throughput manufacturing, and its low modulus may allow change of device dimensions during operation.
2.3 High-throughput fabrication

There are broadly two big advantages in using the fabrication process described. First of all, the method of manufacturing microfluidic devices presented in this document presents an advantage. Hot embossing is a high-throughput and easily-scalable technique, leading to faster and cheaper production. The inexpensive high throughput fabrication of microfluidic devices can yield broad distribution of the devices enabling access to personalized diagnosis, large sample sizes for robust data collection, and high-throughput screening. The second advantage is from the material used in manufacturing the device. Usage of hard plastics instead of PDMS is advantageous because the nature of hard plastic is less hydrophilic than PDMS and not porous. These properties will reduce the undesirable absorption of hydrophobic proteins, which occurred in PDMS devices. There are many types of hard plastics that can be used in hot embossing such as COC, polystyrene, polycarbonate, PMMA, and polyethylene, and their properties are compared in Table 2-1. Among those thermoplastics, COC is our particular interest in fabricating the microfluidic device using hot embossing. Low glass transition temperature allows low working temperature, which can enhance rate of production, and low water absorption and solvent resistance enables wide range of usage of possible experiments. Good optical property is highly desirable since cell culture platform requires access to imaging, and the excellent optical transmissivity of COC suit COC as a prime candidate.
Table 2-1: Summary of physical properties for common microfluidic thermoplastics [18]

<table>
<thead>
<tr>
<th>Polymer</th>
<th>Acronym</th>
<th>$T_{e}$ (°C)</th>
<th>$T_m$ (°C)</th>
<th>CTE ($10^{-6}$°C$^{-1}$)</th>
<th>Water absorption (%)</th>
<th>Solvent resistance</th>
<th>Acid/base resistance</th>
<th>Optical transmissivity</th>
<th>Visible</th>
<th>UV$^a$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cyclic olefin (co)polymer</td>
<td>COC/COP</td>
<td>70-155</td>
<td>190-320</td>
<td>60-80</td>
<td>0.01</td>
<td>Excellent</td>
<td>Good</td>
<td>Excellent</td>
<td>Excellent</td>
<td>Excellent</td>
</tr>
<tr>
<td>Polymethylmethacrylate</td>
<td>PMMA</td>
<td>100-122</td>
<td>250-260</td>
<td>70-150</td>
<td>0.3-0.6</td>
<td>Good</td>
<td>Good</td>
<td>Excellent</td>
<td>Good</td>
<td>Excellent</td>
</tr>
<tr>
<td>Polycarbonate</td>
<td>PC</td>
<td>145-148</td>
<td>260-270</td>
<td>60-70</td>
<td>0.12-0.34</td>
<td>Good</td>
<td>Good</td>
<td>Excellent</td>
<td>Poor</td>
<td>Poor</td>
</tr>
<tr>
<td>Polystyrene</td>
<td>PS</td>
<td>92-100</td>
<td>240-260</td>
<td>10-150</td>
<td>0.02-0.15</td>
<td>Poor</td>
<td>Good</td>
<td>Excellent</td>
<td>Poor</td>
<td>Poor</td>
</tr>
<tr>
<td>Polypropylene</td>
<td>PP</td>
<td>-20</td>
<td>160</td>
<td>18-185</td>
<td>0.10</td>
<td>Good</td>
<td>Good</td>
<td>Good</td>
<td>Fair</td>
<td>Poor</td>
</tr>
<tr>
<td>Polyetheretherketone</td>
<td>PEEK</td>
<td>147-158</td>
<td>340-350</td>
<td>47-54</td>
<td>0.1-0.5</td>
<td>Excellent</td>
<td>Good</td>
<td>Poor</td>
<td>Poor</td>
<td>Poor</td>
</tr>
<tr>
<td>Polyethylene terephthalate</td>
<td>PET</td>
<td>69-78</td>
<td>248-260</td>
<td>48-78</td>
<td>0.1-0.3</td>
<td>Excellent</td>
<td>Excellent</td>
<td>Good</td>
<td>Good</td>
<td>Poor</td>
</tr>
<tr>
<td>Polyethylene</td>
<td>PE</td>
<td>-30</td>
<td>120-130</td>
<td>180-230</td>
<td>0.01</td>
<td>Excellent</td>
<td>Excellent</td>
<td>Good</td>
<td>Fair</td>
<td>Fair</td>
</tr>
<tr>
<td>Polyvinylidene chloride</td>
<td>PVDC</td>
<td>0</td>
<td>76</td>
<td>190</td>
<td>0.10</td>
<td>Good</td>
<td>Good</td>
<td>Good</td>
<td>Poor</td>
<td>Poor</td>
</tr>
<tr>
<td>Polyvinyl chloride</td>
<td>PVC</td>
<td>80</td>
<td>180-210</td>
<td>50</td>
<td>0.04-0.4</td>
<td>Good</td>
<td>Excellent</td>
<td>Good</td>
<td>Poor</td>
<td>Poor</td>
</tr>
<tr>
<td>Polysulfone</td>
<td>PSU</td>
<td>170-187</td>
<td>180-190</td>
<td>55-60</td>
<td>0.3-0.4</td>
<td>Fair</td>
<td>Good</td>
<td>Fair</td>
<td>Poor</td>
<td>Poor</td>
</tr>
</tbody>
</table>

$T_{m}$ melting temperature, CTE coefficient of thermal expansion

$^a$ high UV transmissivity often requires the selection of special polymer grades, e.g. without stabilizers or other additives
Chapter 3

Materials and Methods

3.1 Material selection

Cyclic olefin copolymer (COC) served as the base material of the hot embossed the microfluidic devices. Manufacturers offer several types of COC with different glass transition temperatures, allowing optimal COC material selection depending on device requirements and processing constraints. The COC material used in this study was 2.0 mm thick Zeonor 1060R (Tg 100°C) from Zeon Chemicals (Louisville, KY, USA) as a plate for embossing, and 100 μm thick Topas 8007 (Tg 77°C) from Topas (Tokyo, Japan) as the laminated thin film layer.

3.2 Microfluidic device molding

Hot embossing, schematically illustrated in Figure 3-1, generated the microfluidic devices. A durable master, which can withstand high temperature and pressure, was created from epoxy to hot emboss the COC material. Fabrication of the epoxy master required a series of two molds. The first mold consisted of a 4-inch silicon wafer with SU8 photoresist (MicroChem, MA, USA) layer, patterned using a standard photolithography technique as described in detail previously [27]. Briefly, SU8 was applied to clean, pre-baked silicon wafers, spin-coated at 2000 rpm for 30
seconds twice, exposed to UV light using a mask aligner (Karl Suss MA-6; Suss America, Waterbury, VT), developed for 12 minutes in developer (Shipley AZ400K) and baked at 150°C for 15 minutes, resulting in a 10 μm ± 10 μm height SU8 pattern. The patterned SU8 photoresist served as a mold to create the second mold, a negative replica cast mold made of poly-dimethyl siloxane (PDMS, Silgard 184, Dow Chemical, MI, USA). The PDMS base elastomer and curing agent were mixed in a 10:1 ratio by mass, poured on the patterned SU8 wafer, placed under house vacuum for 30 minutes to degas, and cured in an oven at 80°C for more than 2 hours. The durable epoxy master mold was created by mixing Conapoxy (FR-1080, Cytec Industries Inc., Olean, NY, USA) in a 3:2 volume ratio of resin and curing agent, pouring it into the PDMS mold, and curing it at 120°C for 6 hours.

Figure 3-1: Schematic of hot embossing process

The epoxy master mold formed the microfluidic features in the COC plate through hot embossing using a custom built laminator, which controlled temperature via a thermocouple and heater control system and embossing pressure by applying compressed air and vacuum. The
COC plate was placed on the epoxy master, loaded into the laminator, and embossed at 100 kPa and 120°C, for one hour. The resulting embossed plates were cooled to 60°C under 100 kPa pressure, then unloaded from the laminator and separated from the epoxy master mold. The embossed plates were then trimmed and drilled to create holes to fluidically access the channels. After sonication in ethanol, the COC embossed plates received an oxygen plasma treatment using Technics plasma etcher (Technics Inc., Dublin, CA, USA) for 30 seconds at 100 W intensity and 100 mTorr pressure. To seal the devices, a lamination process bonded a thin COC film to the embossed microfluidic layer. To preheat, the embossed layer was placed on hot plate at 77°C with the thin film COC on top of the plate covering the channels for 20 minutes. After preheating, the embossed plates and film were run between two rollers heated to 120°C for lamination by thermal fusion bonding. After assembly of the device was complete, the devices were sterilized using ethylene oxide (ETO) for 24 hours for cell culture. To facilitate adhesion of the collagen gel to the COC as well as cell attachment within the device, the inner surfaces of the device were soaked in 1 mg/ml poly-d-lysine (PDL, Sigma-Aldrich, St. Louis, MO, USA) coating solution for minimum 3 hours [7].

For cell viability tests, PDMS devices used as controls were fabricated as described in detail previously [6]. Briefly, a PDMS mixture was cured in a mold consisting of patterned SU8 on a silicon wafer, perforated with a biopsy punches to create ports for fluidic access, and autoclaved (20 minutes wet cycle followed by 20/10 minutes dry cycle). Then the PDMS devices and glass substrates were plasma treated with oxygen for irreversibly bonded to seal the fluidic channels, and later coated with PDL solution.
3.3 Metrology

3.3.1 Scanning electron microscopy (SEM)

The microchannel features of the SU8 on silicon molds, PDMS molds, and epoxy master molds and embossed COC devices were observed using scanning electron microscopy (SEM). All samples were cleaned using nitrogen gas to remove dust particles, mounted to SEM stubs using carbon tape, then sputter coated with 50 angstroms of gold in argon plasma under vacuum. The images were captured with 5kV acceleration using a Hitachi S-3500N (Tokyo, Japan).

3.3.2 Profilometry

The profile of epoxy master and embossed COC device were traced using a contact profilometer (P-16 KLA-Tencor, Milpitas, CA, USA) with a triangular tip prob. The samples were cleaned with nitrogen gas prior to the scan. The scan speed was 50 μm/sec with a 50 Hz sampling rate. The linear scan was performed over length of 0.5 mm sections per scan for each sample.

3.3.3 Three-point bending tests using Dynamic Mechanical Analyzer (DMA)

The strength of the bond between the embossed plates and laminated thin film created during the thermal fusion process was evaluated by a three-point bending test using a dynamic mechanical analyzer (Q800 DMA, TA Instruments, New Castle, DE, USA). A 55 mm (L) x 12 mm (W) x 2 mm (thick) COC plate test sample base plate was created to fit the dimensions of the three-point bending test and bonded to a 50 mm (L) x 4 mm (W) x 100 μm (thick) COC film using the procedure described above. For each test group, different plasma treatment conditions were applied to the materials before bonding. “All plasma” indicates plasma treatment to both COC
plate and film, "half plasma" indicates plasma treatment only to the COC plate, and "no plasma" indicates no plasma treatment prior to bonding. After thermal bonding, another test sample base plate piece was bonded to the previously thermally bonded film-plate sample by adhesive carefully applied to the film using a sharp utensil to spread the adhesive uniformly as thin layer and restrict adhesive to the film area. The samples were dried for 24 hours before testing to ensure that the adhesive was completely dried. The final configuration of the test sample consisted of two 2 mm thick COC plates sandwiching the thin film, with one side having a thermally bonded plate-film bond and the other side having an adhesively bonded plate-film bond. In the three-point bending test, the displacement of the sample was recorded as load increased at rate of 2.0N/min up to maximum load of 18N. During load ramping, a discontinuity in displacement versus load output was observed as the bond failed. The Student t-test compared no-plasma versus half-plasma and no-plasma versus all-plasma with p-values less than 0.03 considered significant.

![Figure 3-2: Schematic of DMA testing process](image_url)
3.3.4 Contact angle measurement

Contact angles of distilled water on COC plate were measured before and after surface treatment using an optical goniometer (Model 190, Rame-hart instrument co., Netcong, NJ, USA) by sessile drop method. Water droplets of 10 μl in volume were released from a syringe above the sample surface, and the images of droplet formation captured by high-resolution camera were analyzed using image analysis software (DROPImage, Rame-hart instrument co., Netcong, NJ USA) to calculate the contact angle. For each time points, minimum of 3 locations on each sample were tested and contact angles were averaged.

![Figure 3-3: Schematic of sessile drop method](image)

In order to find the change of hydrophilicity as function of plasma treatment duration, the contact angles measurements were recorded for each duration points 10 minutes after each treatment. In order to quantify the recovery of hydrophilicity over time, contact angles were measured at different time points starting before plasma treatment (-0.3 hours), after plasma treatment (0.0 hours), and over time for a period up to 168 hours (remaining time points). The samples were placed in petri dish and in vacuum during the experiment. The effect of thermal treatment of COC on hydrophilicity recovery over time was also shown by repeating the above
process to COC sample that was heat-treated. “COC heated” samples were first heated at 77°C for 30 minutes and later at 120°C for 2 minutes after plasma treatment, and the contact angle measurement (0.0 hours) began soon after completing the heat treatment.

### 3.4 Cell culture

The protocol for human microvascular endothelial cells (hMVECs) culture was identical to that described previously [4]. In brief, hMVECs (Lonza, NJ, USA) were cultured in endothelial growth medium (EGM-2MV, Lonza, NJ, USA), and maintained at 37°C and 5% CO₂. The medium was changed every two days until an 80% confluent monolayer was formed before passaging or seeding. To seed samples, hMVECs were detached with trypsin (Invitrogen, Carlsbad, CA, USA) and resuspended in the same medium at a cell density of 2 million cells/ml. A 40 µl volume of cell suspension was introduced to PDL-coated PDMS and COC devices for a minimum of 3 hours, washed using deionized water, and dried in an oven at 80°C for minimum of 24 hours under sterile conditions. Collagen type I (BD Biosciences, San Jose, CA, USA) gel solution with 2.0 mg/ml density was inserted in the gel region of the device using 10 µl pipet and were incubated in humidity box to be polymerized, and the same medium was inserted to the rest of the channels. Then hMVECs were cultured for the prescribed amount of time. All experiments were conducted using cells of passage 8 or lower.

A live/dead viability.cytotoxicity kit (Invitrogen, Carlsbad, CA, USA) was used to assess cell viability. In live cells, the intracellular esterase activity transformed calcein AM to fluorescent calcein, resulting in green fluorescence. In dead cells, the entry of ethidium homodimer-1 (Ethd-1) into damaged membranes of dead cells and subsequent binding to nucleic acid resulted in red fluorescence. The samples were washed with Hank’s balanced saline solution...
(HBSS), and incubated for 15 minutes with 500 μl HBSS solution containing 1 μl calcein AM solution and 1 μl EthD-1 solution. The Fluorescent microscope images were acquired with a microscope and a Nikon TEH100 camera and OPENLAB 4.0.4 software, and processed using ImageJ software (National Institute of Health, http://rsb.info.nih.gov/ij/) to false-color the gray scale images. The total cell population was quantified by counting the total number of green and red cells, and the percentage of live cell population was reported.
Chapter 4

Results and Discussion

4.1 Replication

The durable epoxy master mold used in the hot embossing process enabled molding of numerous microfluidic devices with good feature replication. A single epoxy master generated over 20 samples without degradation. Release of the device plate from the master required no additional special techniques or solvents. The process steps conveniently produced high quality replicas of the original microfluidic design pattern. Figure 4-1 (a) shows the replicated pattern configured in 4-device arrays at each step in the mold and device fabrication process. The images on the left side show 100 mm diameter molds which were either a positive or negative relief replica of the original microfluidic pattern in a different material. Cautious handling of PDMS mold was necessary during the replication step because the PDMS mold required a flat support to supply sufficient rigidity during the epoxy casting step and ensure planarity of the resulting epoxy mold.

Inspection of the microfluidic device features confirmed the quality of replication. Figure 4-1 (b) shows scanning electron microscope (SEM) images of the post and channel structure of a device to the right of the corresponding full mold images. A single device schematic with expanded gel region indicates the SEM-imaged area with a dashed line. Since the area contained
both a protruding post and recessed channel, it served as an excellent region to gage replication quality for these two feature types within the device. All the surfaces of masters and embossed device were uniform without any rough finish based on SEM observation.

Profilometry quantified feature depth and surface finish for the epoxy master and the final embossed COC plate, indicating good replication accuracy during the hot embossing step. Table 4-1 shows profilometry data for height and roughness of features for scans taken across the gel-region channel. Feature heights were $110.16 \pm 9.56$ µm for the epoxy master and $109.30 \pm 10.66$ µm for the COC plate. The roughness of mold and plate as measured by profilometry were $1.01 \pm 0.11$ µm and $1.17 \pm 0.19$ µm respectively, which agrees with observations from SEM images and indicates no irregularity, coarseness, voids or other artifacts of incomplete replication during the hot embossing step. The differences in height and roughness of the channel between epoxy master and COC plate were within the standard error. Feature height measurements taken at various locations on both epoxy mold and embossed device were uniform across the device area.

Table 4-1. Profilometer results for the epoxy master and COC plate confirm accurate hot embossed replication of the microfluidic structure in COC devices

<table>
<thead>
<tr>
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<th>Height (µm)</th>
<th>Ra (µm)</th>
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<tbody>
<tr>
<td>epoxy master</td>
<td>110.16 ± 9.56</td>
<td>1.01 ± 0.11</td>
</tr>
<tr>
<td>COC plate</td>
<td>109.30 ± 10.66</td>
<td>1.17 ± 0.19</td>
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The results indicate pattern replication by hot embossing process produces accurately formed devices with minimal surface defects. Using the fabrication process described above, we
can produce a durable epoxy master for hot embossing. The fabrication process of an epoxy master does not have to be limited to this particular method mentioned here, and a hot embossing master could also be made of a different material such as micro-milled brass or electroformed nickel [29, 30]. The fabrication of microfluidic devices using hot embossing is advantageous from its higher rate of production than soft lithography method for PDMS devices, and is mass-manufacturable yet still a flexible process [31].
Figure 4-1: Products of each replication step of the process demonstrating good replication of features.
4.2 Bond strength

Laminating a thin COC film to the embossed microfluidic structures resulted in a sealed device with controlled laminated layer bond strength. Figure 4-2 illustrates the double roller laminator thermally bonding the thin COC film to the device resulting in a device with sealed fluidic channels. Properly bonded areas were easily distinguished from non-bonded areas by visual. In addition, green dye introduced into the channels remained within the sealed structures as shown in Figure 4-3, further confirming proper sealing of the fluidic channels by the laminated layer.

Figure 4-2: Schematic of thermal bonding process using double rollers.
The three-point bending tests using a DMA determined the strength of the thermal fusion bond between the thin COC film and COC plates created by the lamination process. The displacement versus force plot for each sample possessed a discontinuity at the point when the thin COC film laminate bond fails, illustrated by a representative plot in Figure 4-4. The bond strength was determined by the shear stress calculated from the load occurring at laminate bond failure.
The bond strength depended on the plasma treatment applied to the COC components before lamination. "All plasma" samples received plasma treatment to both the COC plate and thin film, "half plasma" samples received plasma treatment only to the COC plate and not the film, and "no plasma" samples received no plasma treatment prior to bonding. The load at the discontinuity of the displacement versus force plot was used to determine the critical load at delamination and used to calculate the shear stress at failure, assuming the thermal bond would fail before the adhesive bond. This assumption was proved correct from observation after the test when the bonding by adhesive was still intact and the thermal fusion bond was no longer intact. The stress levels were $252.3 \pm 18.6$ kPa for no plasma, $414.0 \pm 74.5$ kPa for half plasma, and $685.6 \pm 47.2$ kPa for all plasma as plotted in Figure 4-5. Half plasma and all plasma data were compared to no plasma data, and both pairs were found to be statistically significant with p value lower than 0.03.
The three point bending test mentioned above used sandwich-structured samples as described previously. The sandwich-structured sample configuration located the thermally-bonded section near the neutral axis of the sample. Since transverse shear stress is highest at the neutral axis of a bending beam, the sandwich-structured sample used the load efficiently to delaminate the bond. This allowed observation of delamination point with load below 18N, and therefore improved the accuracy. While delamination stress level was calculated from three-point bending using DMA in this paper and others, it is possible to use different methods to quantify the level of bonding such as peel test or crack test, which quantify force or energy to break the bond [36-39].

4.3 Collagen gel retention and placement

Oxygen plasma treatment created controlled hydrophilicity on COC devices which enabled control of collagen gel location in the devices. Hydrophilicity as measured by water contact
angle, depended on plasma treatment duration at a fixed plasma intensity of 100W as shown in Figure 4-6. Without plasma treatment, the COC material was almost hydrophobic with a contact angle $84.5 \pm 3.6^\circ$. In contrast, after a plasma treatment as short as 10 seconds, the COC material became hydrophilic with a contact angle of $29.7 \pm 1.2^\circ$. For longer durations of plasma treatment, the resulting hydrophilicity increased as measured by decreased contact angle measurements, although not to the extent of the increase seen due to the initial plasma treatment.

![Figure 4-6: Variation of contact angle for given plasma duration.](image)

The hydrophilicity change due to plasma treatment was more stable over time for COC devices versus PDMS devices. Figure 4-7 shows the contact angle change over time after plasma treatment. Contact angles before 30 seconds at 100W intensity plasma treatment (-0.3 hours), after the plasma treatment (0.0 hours), and over time following the plasma treatment (remaining time points) indicated the long-term stability of COC surface energy compared to PDMS. The PDMS rapidly returned to an almost hydrophobic state after plasma treatment induced a
hydrophilic condition. Within the first hour after plasma treatment the contact angle on PDMS recovered from $11.2 \pm 2.9^\circ$ to $50.8 \pm 4.8^\circ$, and by the third hour the contact angle was $80.0 \pm 3.1^\circ$. In contrast, the COC material maintained a hydrophilic state for several days after plasma treatment with the contact angle remaining below $40^\circ$ for 24 hours and only reaching $50.6 \pm 0.5^\circ$ after 168 hours. Thermal treatment of the COC increased its hydrophilicity after plasma treatment, although the hydrophilicity remained more stable than that of PDMS. The contact angle for heated COC was $53.5 \pm 1.7^\circ$ at 24 hours after plasma treatment, and remained below $65^\circ$ for 168 hours. Both time and thermal treatment of COC altered hydrophilicity after plasma treatment, although the changes occurred over a long timescale with the material remaining hydrophilic through the conclusion of data collection. This indicates that a lower contact angle can be preserved longer on COC than PDMS, which could be an advantage for cases needing higher level of hydrophilicity for longer period of time.

Figure 4-7: Contact angles before plasma treatment (-0.3 hours), after the plasma treatment (0.0 hours), and over time following the plasma treatment (remaining time points) indicate the long-term stability of COC surface energy compared to PDMS.
Controlling hydrophilicity of the COC device enabled gel containment as shown in Figure 4-8. The red markers are in the channels and point to the gel/channel interface. The three channels were separated by collagen gels inserted in two gel regions up to the square posts, with the gel contained by surface tension. Collagen gel was inserted using a pipette so as not to overfill the gel region. For COC devices not thermally treated, gel spread to then entire device immediately upon insertion and was not restricted by the square posts. However, for COC devices with more hydrophobic surfaces, either due to shorter plasma treatment or longer post-plasma treatment thermal treatment, gel spread predictably up to the square posts.

The collagen gel mimics an in vivo extracellular environment in this COC microfluidic device, and is a key feature of the device to culture and observe cells in 3D. There has not been a study (up to our knowledge) that collagen gel is inserted in hot embossed COC device. It is also important to note that different level of hydrophilicity resulted from different level of surface energy effects cells to behave differently in signaling or differentiating manner, that being able to control the hydrophilicity within a device enables better control of cell experiments. From this
result of modifying hydrophilicity and enabling gel containment, it is shown that all COC devices can fully function as a microfluidic device for cell culture platform.

4.4 Cell viability

Cell viability after 72 hours of culture in COC devices was similar as compared to PDMS devices and standard polystyrene (PS) well plates. Quantified live/dead assay data for hMVECs showed near 80 percent cell survival rates for cells culture in COC devices, PDMS devices, and standard PS well plates as shown in Figure 4-9. Green fluorescent signal indicated active (live) cells that are undergoing intercellular esterase activity and red indicated permeable (dead) cells as shown in Figure 4-9. Cell culture conditions included no perfusion of media other than a daily change of media, standard incubator conditions as mentioned previously, and no pre-treatment of the media. The percent cell population data plotted was the average value of \( n = 3 \) replicates for each condition with the standard error of mean shown. A paired Student t-test showed no statistical difference for viability rates in PDMS and COC. As seen in Fig 5b, the optical clarity of COC device was also comparable to PDMS device.
Figure 4-9: Percent of live cell population for polystyrene (PS) well plate, PDMS device, and COC device (a), and fluorescent image showing live (green) and dead (red) cells in PDMS and COC devices (b).

This test indicated that COC devices could indeed be used in place of PDMS devices for hMVECs culture and experiments. One possible concern regarding transition from PDMS to COC device may be inherent low oxygen permeability of hard plastics, and some tried resolving this issue by combining both PDMS and hard plastic [40]. However, this result confirms that cells are capable of surviving for minimum of 72 hours in COC devices with nothing more than normal daily change of media.
Chapter 5

Conclusion

We have presented the fabrication methods for a microfluidic device using COC for cell culture platform. A hot embossing process with epoxy masters can replicate features well on to the COC plate, and thermal bonding can laminate the thin COC film to the embossed COC plate when combined with plasma and thermal treatment, completing the COC microfluidic device. The combination of plasma and thermal treatment also enables control of the hydrophilicity level of the COC plate such that collagen gel which mimics the extra cellular environment can be inserted in the device and contained for the proper function. Finally, the cell viability in the COC device has been confirmed. Therefore, the transfer of the microfluidic system to the COC devices is now complete, and now we have a new microfluidic system for cell culture platform that can be produced in cheap and high-throughput manner.
Appendix A

Fabrication of PDMS Microfluidic device

1. Prepare PDMS with 10:1 ratio of polymer resin and curing agent by weight. Total 40g of PDMS is necessary for one 4-inch silicon wafer with 4 device SU8 pattern.

2. Stir PDMS mixture well with plastic stirrer. When thoroughly mixed and is blurry, put the cups containing PDMS mixture into vacuum chamber and turn vacuum on. Turn off the vacuum after brief suction, and leave it as is for 30 minutes.

3. Place silicon wafer on the scale and add 40g of PDMS mixture on top of the wafer. Place the wafer and PDMS back into the vacuum chamber for another 30 minutes in vacuum. Keep the vacuum on.

4. Place silicon wafer and PDMS mixture in the oven at 80°C for overnight, and minimum of 2 hours.

5. Trim out PDMS from the wafer using scalpel.

6. Punch out each device using 1” diameter pipe-punch.

7. Punch out 4mm fluid access ports using biopsy punch.

8. Punch out 1.25mm gel injection hole using biopsy punch.
9. Use 3M magic tape to clean up the patterned surface and sides. Use the same tape to cover up the surface when storing is needed.

10. Autoclave the devices for both wet and dry cycle. In wet cycle, place devices in DI water, and run 20 minutes of sterilization and 10 minutes of drying cycle. Take the devices that have underwent wet cycle, and place them in autoclavable containers and run dry autoclave cycle consisting of 20 minutes of sterilization and 10 minutes of drying cycle. After autoclaving, place the devices in petridish, and store them in oven until using them.

11. Cool down for 30 minutes in the hood.

12. Clean plasma etcher area. Place 6 steril devices and 6 circular cover slips (d= 35mm, t=100 μm) in plasma etcher. Close the valve, turn on vacuum for 2 minutes, and turn on plasma. Start from low to medium to high, and stay at high level for 45 seconds and check for bright purple color before turning plasma off in the reverse order. Try to maintain purple during 45 seconds of plasma.

13. Apply poly-d-lysine surface coating solution (1mg/ml) to plasma treated devices via gel insertion port. Apply 80 -100 μl per device, and leave it for minimum 3 hours.

14. Aspirate out coating solution, and wash the channels of the devices with DI water by taking about 150 μl of DI water. Wash twice for each device. Place the devices in oven (80°C) for minimum 24 hours. The devices are now ready to be gel filled.
Appendix B

Fabrication of COC Microfluidic device

B.1 Fabrication of Epoxy Master

1. Make PDMS mold with wall.
   Do all following steps in the hood.

2. Perform surface treatment for PDMS mold.

3. Prepare conapoxy (Fr 1080) mixture A : B = 3 : 2 by volume or A : B = 100:83 by mass.
   By volume: Using 10 mL syringe in a cup, prepare appropriate volume estimating about 30mL/wafer. (A : B = 18mL : 12mL)

4. Place PDMS sample on 4” metal spacer, and pour 30mL conapoxy per PDMS sample.

5. Remove the bubbles in the mixture by placing the PDMS mold in the oven (120°C) for about 10min.

6. Most of the bubbles should be removed, but there may be still bigger bubbles left.
   Remove the bubbles using pipet tips. Make sure there are no bubbles at the pattern surface.
7. Once all bubbles are removed, place 5” metal weight on top of PDMS wall.

8. Place in oven 120°C for 6 hours.

B.2 Embossing

1. Have epoxy mold, plastic sample, thin PDMS layer covered aluminum spacer ready.

2. Clean the mold and plastic surface and tape together the 2 opposite corners of the mold and plastic stack to fix them.

3. Turn on the temperature to 120 °C

4. Place in the embosser from bottom up:
   a. Epoxy mold
   b. Plastic
   c. PDMS
   d. Al spacer

5. Insert the release valve on the port of the embosser so that air can go through the embosser.

6. Place the top cover of the embosser by hand while making it as flat as possible.

7. Remove the handle on the top cover.

8. Detach the release valve, and insert the vacuum valve to the same port, and turn on the vacuum.

9. Notice the vacuum pressure is 25inHg on the gage, and turn off the vacuum and detach the valve so that nothing is attached to the port on the embosser. The pressure should be maintained at 25inHg.
10. Assemble the outer top lid of the embosser using the 4 hex bolts.

11. Attach the air valve from the wall to the top lid and turn on the air pressure to 20psi.

12. Wait for 60 minutes.

13. Turn off the temperature control, and wait for the embosser to cool down much under the glass transition temperature of the plastic (100°C for zeonor). The cooling may take about an hour by natural convection.

14. Turn off the air pressure, and disassemble the top lid.

15. To remove the top cover, insert the air pressure valve to the port on the embosser, and turn on the air slowly until the top cover pops out.

16. Slowly detach the embossed plastic from the mold.

B.3 Thermal bonding


2. Cut COC thin film to the size of embossed COC plates using scissors.

3. Plasma treat embossed COC plates for 30 seconds at 100W intensity with embossed side facing up.

4. Gently place the film on top of the plasma treated COC plates, and wrap aluminum foil out of the plate and film combo.

5. Heat up the plate and film combo with aluminum foil on hot plate at 77°C for minimum 30 minutes. Place some heavier weight, such as Al stock material to press down the sample on to the surface of hot plate. The film should be placed near the hot plate surface such that the order of entire sample from bottom touching the hot plate surface should be Al foil $\rightarrow$ COC film $\rightarrow$ embossed COC plate $\rightarrow$ Al foil $\rightarrow$ Al weight.
6. While heating on hot plate, turn on the heater of roller to 120°C.

7. After 30 minutes on hot plate, quickly transfer the sample to roller for lamination.

8. Place a metal plate such as copper or aluminum that is 1 to 2 mm thick to give support to the sample while being fed in to the roller. Place the metal plate underneath the film side of the sample.

9. Run the rollers at 0.2 to 0.3 m/sec (minimum speed of the equipment) and feed in the sample. If the rollers become stalled at low speed, start with higher speed (0.6 m/sec) to feed in the sample, and quickly ramp down the speed to 0.2 m/sec once the sample is fed in.

10. Repeat step 9 for three more runs to ensure complete bonding.
Appendix C

Three-point bending process using DMA

The following process is inserted in command line for three-point bending test using DMA.

1. Data storage off
2. Equilibrate at 26.00 °C
3. Isothermal for 0.20 min
4. Data storage on
5. Ramp force 2.0000 N/min to 18.0000
Bibliography


