Design and Fabrication of Micro-Structured Surfaces for Algal Biofilm and Human Cell Migration Studies

by

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Department of Electrical and Computer Engineering
University of Toronto

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Abstract

Cells have been reported to respond to structured surface cues that are on the micro and nanometer scale. In this work, widely known semiconductor fabrication processes such as lithography, etching along with hot embossing methods were utilized to fabricate consistent micro-patterns in poly(methyl methacrylate) (PMMA) substrate in order to grow algal biofilms in a photo bioreactor. Results show that productivity of biomass has doubled on deep (~20 µm) V-grooved PMMA surfaces compared to smooth and shallow (~1.5 µm) grooved PMMA surfaces. The hot-embossing parameters such as force, temperature and wait-time settings in Jenoptik HEX 02 hot embosser were tested and optimized for an efficient pattern transferring process using PMMA sheets and PMMA resist. Furthermore, micro-structured surfaces on silicon substrates were fabricated with distinct flat-groove interface for guided cell migration study and the custom-made Si stamp design helped in concluding that cells were guided on the interface through mechanical exclusion interaction.
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Chapter 1
Introduction

1 Introduction

1.1 Background

The increase in world population has triggered a rapid increase in energy demand around the
Administration, energy consumption in the year 2010 was 524 quadrillion Btus (British thermal
units) and is expected to increase by 56% over the next 30 years with a projected consumption of
820 quadrillion Btus in 2040 (Fig. 1) when all the countries around the world (Organization for
Economic Cooperation and Development [OECD] countries and non-OECD countries) are taken
into consideration [1].

Fig. 1: World energy consumption between years 1990-2040 (Source: U.S. Energy Information Administration (July 2013)) [1].

To meet this high demand for energy over the next three decades, fuel input in power generation
will be a mixture of many sources of energy with an increasing prospect for renewables and a
decreasing dependence on non-renewable sources [2]. Biofuel is a renewable energy source and
it is a liquid fuel converted from biomass to meet our energy needs, particularly transportation.
These are traditionally derived from living or recently living organisms such as plants and plant
derivatives. Unlike fossil fuels, which are non-renewable and release CO₂ thereby negatively
impact the environment, there is a lot of attention being given to biofuels and they are slowly
becoming an integral part of a cleaner source of energy production. Although CO\textsubscript{2} is released while producing biofuel it can be used to capture CO\textsubscript{2} from atmosphere during growth. In another three decades, among the different methods to produce non-petroleum liquid supplies, biofuel production will have a considerable increase [1]. It is believed that interest for renewable energy sources, possible economic interest and technological advancement can make this into a reality.

However, biofuels for the most part are being produced from corn, sugarcane, soybeans and wheat in the United States, Brazil and Canada [3]. Just like there is demand for energy, there is also a high demand for food to meet the needs of increasing world population. Therefore, using natural food and agricultural land for biofuel production is under scrutiny. Furthermore, biodiesel produced from oil crops, waste cooking oil and animal fat cannot efficiently produce the oil content needed for global transportation fuel demands. These concerns have raised interest in developing second-generation biofuels from non-food feedstock such as microalgae.

1.2 Motivation

Microalgae have been a potential source of biofuel because of their high oil content and ability to quickly produce biomass [4]. Microalgae for the most part are grown in suspension inside raceway ponds and open-pond reactors. These methods require large amounts of water and energy input [5]. After the formation of algal biomass in suspension, more energy will be required to harvest and dewater them to produce usable biofuel. On the other hand, growing them as films can potentially be an economically favorable option because large concentrations of biomass can be collected at one place, making it easier to separate them in a single bio reactor [6, 7]. Additionally they can be grown on non-agricultural landscape where there is an abundance of sunlight. Growing biofilms has more advantages like beneficial genes are expressed in high concentration, nutrients can be accessed easily and a large amount of extracellular polymeric substance (EPS) can be produced in this way [8].

However material surface properties can affect biofilm growth when they are grown on surfaces. Many studies have proved that cells of any organism respond profoundly to surfaces and chemical or physical cues play an important role in directing them and eventually growing them on surfaces [9, 10, and 11]. While patterning the surface with chemical cues with active molecules however can influence the cells by secreting substances thus shortening their lives.
This makes the physical cues to play an important role in tissue engineering and microorganism studies where a lot of attention has been given in micro and nano structuring of surface to provide cues to the cells. Much of this research in animal cell growth and tissue engineering on various micro/nano structured surfaces have revealed in interesting behaviors of cells and tissue formation [12, 13]. On the other hand, in microorganism study, some specific micro patterning has been successfully made for detaching algae from marine surfaces with an end result of utilizing surface texturing for anti-fouling purposes [14, 15, and 16].

Since anti-fouling research had got the attention of micro texturing, very little is known about getting them to grow on micro-structured surfaces. Having the tissue engineering and bacterial biofilm research backgrounds and knowing the need for algal biofilm formation for biofuel purposes, now is the time to study the different patterned surfaces that can influence algal biofilm formation. Through micro-patterning the surface, a range of hydrophobic surfaces can be made and these surfaces can be tested to study the film’s interaction and surface suitability for higher productivity of the film.

Irving, T, has noticed in his preliminary study that altering hydrophobicity and increasing the surface-to-volume ratio may influence the initial algal biofilm attachment which may also mean increasing the total biofilm mass [6, 17]. Micro-patterning increases the surface-to-volume ratio by providing more surface area for attachment and results in higher productivity of the film. Therefore understanding the interaction between the algae and the micro-patterned surface becomes important in order to design and optimize a biofilm-based photo bioreactor.

Micro-structured surfaces are in general made in a hard master and then transferred into compatible and inexpensive polymers using soft lithography process. Hot embossing is one method used in replicating patterns from a hard stamp into a soft material like polymer sheets and this method has been widely utilized in many studies over the years. This method is mainly suitable for producing micrometer scale replicas. Although there are some standard embossing recipes available for specific polymer embossing, only very little information is recorded on the parameters suitable for embossing needs that utilize different kinds of master stamps (brittle to hard) using specific embossing machines. Many processes have been done with hard master like nickel and a trial-and-error process is what many researchers used to identify the parameters and setting for their specific needs. Some simulation results are published using home-made and lab-
made embossing machines as well to give an idea on how the polymer material flows under certain force and temperature conditions [18]. Apart from the parameters related to the machine and the substrate being embossed into, factors related to the master and the size of features on the master can influence the embossing procedure as well. Therefore here we attempt to develop a process for using a Jenoptik HEX 02 hot embosser machine for the needs of algal biofilm growth. In doing so we try to optimize and generalize the process for similar needs using this machine now available at U of T.

Designing and fabricating micro-structured surfaces have other applications such as studying animal cell guidance and migration. Guidance and response of individual cells to topographical cues has been extensively studied but very little is known about the response of groups of cells to topographic cues. Collective cell migration is the fundamental process for many cell and tissue progression such as embryo development and adult organ regeneration [19]. Understanding the groups of cells’ response to micro-structured surfaces therefore helps develop knowledge on how these cells behave in general to topographical cues vs. flat surface.

1.3 Objective

The objective of this work is to optimize the hot embossing process using a Jenoptik HEX 02 machine now available for use at the University of Toronto, with a view to developing a fabrication protocol which will allow for the fabrication of a range of micro-structured surfaces for the control of cell growth. For this purpose, micrometer features will be designed, fabricated and tested on silicon-based master stamps and poly(methyl methacrylate) (PMMA) based embossed substrates using semiconductor fabrication processes such as lithography, etching along with hot embossing method.

After optimizing the fabrication procedures, consistent micro-patterns in PMMA substrates are fabricated in order to grow algal biofilms in a photo bioreactor. For this study we collaborated with Scott Genin, PhD candidate (supervised by Professor Grant Allen) in the Department of Chemical Engineering and Applied Chemistry who tested these substrates inside a photo bioreactor and examined them for productivity of algal biomass. The fabricated coupons were inserted in the photo bioreactor and the inoculum contained seven algal species: *S. obliquus* (CPCC 157), *C. vulgaris* (CPCC 147), *Coccomyxa sp.* (CPCC 508), *Nannochloris sp.* (CCAP 251/2), *Nitschia palea* (CPCC 160), *Oocystis sp.* (CPCC 9) and *Oocystis polymorpha*.
As another application to micro-structured surfaces, micrometer features are designed and fabricated on silicon stamps for collective cell migration study in another collaborative work with Camila Londono, PhD candidate (supervised by Professor Alison P. McGuigan) in the Department of Chemical Engineering and Applied Chemistry. Human retinal pigment epithelial cells (ARPE-19) and human foreskin fibroblast (BJ) cells at both sparse and confluent densities were tested on surfaces replicated from custom-made Si stamps.
Chapter 2
Literature Review

2 Literature Review

2.1 Micro-structured surfaces and cells

Cells of any organism move on a surface by locally extending their membrane. These extended membranes attach to their surroundings by exerting a force and subsequently growing on the surface. Therefore cell adherence and its reaction have been identified to be dependent on surface topography. It has been proved in many studies over the years that cells respond profoundly to surface cues whether these cues are chemical or physical [10]. Since chemical patterning of surfaces with active molecules could influence cells to secrete substances thus shortening their lives, physical (i.e. topographical) cues play an important role in human cell engineering and microorganism studies. Topography on the micro- or nanometric scale can be employed in cell engineering, which allows positioning and proliferation of cells [20]. Cell sizes are typically on the order of 1-100 µm and they typically interact with physical features that are on the same scale or smaller, which is why many studies have been done with feature depths that are on the same scale [21, 22].

Using these different techniques, micro and nanometer topographies have been extensively studied in extracellular matrix growth in a particular direction to achieve highly aligned cell culture for future tissue engineering applications. In such a study, epithelial cell alignment was induced by anisotropic groove and ridge topography. Cells align on anisotropic ridges as small as70 nm width, 400 nm pitch and 600 nm groove depth. Lamellipodia cells bridge the grooves at given dimensions and filopodia cells frequently align to the anisotropic direction, proving topographical sensing of the cells [13]. In an earlier study by Clark, P. et al. in 1990, topographical guidance of three different cell types (BHK, MDCK and chick embryo cerebral neurons) were examined on grooves with varying dimensions (4 – 24 µm repeat and 0.2 to 1.9 µm depth). They determined that different cell types interacted differently to the topographical cues [9]. Similarly nano meter scale patterns were fabricated and tested for cells’ response to cues in that scale. Gadegaard et al. studied regular nano-pits which were fabricated using E-beam lithography and transferred onto a glass surface using hot embossing. These topographical cues
have been identified to play an important part in cell engineering (Fig. 2) but pattern fabrication and transfer are limited to small surface areas [20].

Fig. 2: SEM micrograph of fibroblast cell sensing nano pitted surface. Reprinted with permission from © 2006 Elsevier [20].

Another major area that these structured surfaces are employed is in studying the behavior of cancer cells on topographical cues. It has been identified that vascular endothelial growth factor (VEGF), which is over-expressed in malignant breast tumor cells, decreased when cells were grown on 23 nm featured surfaces. Further studies have been directed towards growing these types of cells on even smaller dimensional topography to better understand this behavior [23].

Although the studies of growing animal cells on structured surfaces were started first, they were later extended to studying algae growth and their behavior on patterned surfaces. More importantly, structured surfaces were designed and optimized to detach algal growth on marine surfaces. Biofouling is defined as an undesirable growth of microorganism in a marine environment or industrial water systems. Biofouling creates significant effects such as an increase in flow friction in ships and consumes 40% more fuel to maintain the same speed of a ship that has no fouling [24]. Mechanical detachment, antibiotics, biocides and cleaning chemicals, and non-adhesive surface engineering can remove these biofoulers. The first two methods have downsides, however; mechanical scrapping is not possible in all cases and the current cleaning chemicals are recognized as toxic and therefore can cause adverse effect to the water and environment. Therefore the main method being widely explored is surface engineering with physical topographical cues. Some micro and nano structured surfaces can prevent the settlement of microorganisms when they are engineered with lower surface energy and lower wetting properties. One of the earlier studies on topographical inhibition of marine alga reported
that a Sharklet AF™ surface containing 2 μm wide rectangular periodic features in differing lengths spaced at 2 μm reduced the Ulva settlement by 86%, as can been seen in Fig.3C[15].

![Fig. 3: Ulva settlement on (A) smooth surface;(B) 5 µm wide, 5 µm spaced, and 5 µm high channels and (C) 4 µm high Sharklet AFTM in PDMSe. Scale bars=25 µm. Reprinted with permission from © 2006 Taylor & Francis [15].](image)

J.F Schumacher et al. studied engineered roughness indices of different micro structural topographies and compared them with spore density [14]. All structured topographies showed relatively less spore density compared to smooth surface of PDMS; however, highest roughness index topography (Sharklet AF™) resulted in the lowest spore accumulation. Another study by C-H Choi, suggests that surfaces with sharp-tip tall nanostructures (10 nm radius, 50-500 nm height) also inhibit the microorganism growth; this has been proven with smaller cell populations and weaker cell adherence in both hydrophilic and hydrophobic conditions [24]. Although this suggests potential application in antifouling, anti-microbial applications, the cells studied in the research was human foreskin fibroblast and mouse fibroblast cells on Si based substrates and Teflon. Specific application therefore may depend on the actual material properties of the surface in addition to its effect on micro structuring.

Structured surfaces therefore can be engineered to promote and control algae growth but very little is known about this because of the demand in anti-fouling applications. M.L. Carman et al. suggest that if the topography is engineered to expand its wetting property, the organism may be induced to align with the topographical spacing. This can be done in the same way that rat dermal fibroblast cells were oriented on 0.5 μm high microgrooves with widths from 2 to 10 μm [15, 25].

Bacterial biofilms were also studied on structured surfaces and, in particular, Perni et al reported that micro-patterning with conical features and specific dimensions (25 and 30 μm diameter)
fabricated on silicone exhibited high levels of area covered by bacteria and that the distance between cones did not affect bacterial adhesion [26]. Hydrophobic and hydrophilic material surfaces along with the particular species can influence the attachment and formation of biofilms [27]. It has been suggested in some studies that altering surfaces may also influence the formation of algal biofilms as in the tissue engineering field [15].

As another area of application, single animal cells have been studied for contact cell guidance on grooved substrate but very less has been done in the area of directed migration of groups of cells. Groups of cells coordinate their behavior while maintaining tissue integrity therefore they become an important part in ensuring proper tissue organization. Cells’ local microenvironment gives chemical and mechanical cues in directed migration of cells. When cultured as a single cell, the groove dimension, specific cell type, and their organization have been previously studied but little is known about groups of cells’ response in particular when they are in a confined space with no free space available to move around. In this context, they will respond to such situation by contact guidance of neighboring cells but it is a more complex process because cytoskeletal coordination between the neighboring cells has to match with the cytoskeletal organization induced by grooved surfaces on the cells [19, 30, and 31].

2.2 Fabrication of structured surfaces

2.2.1 Stamp Fabrication

To study cell adherence, progression and other cell behavior on micro- and nano- structured surfaces, number of different processing methods have been investigated. The methods to fabricate the stamp include polymer phase separation, biomolecule replication, photolithography, E-beam lithography, dip-pen lithography, laser irradiation, capillary lithography, X-ray lithography, interference lithography, block copolymer lithography, nanoparticle or colloidal lithography, chemical vapor deposition of carbon nanotubes (CNT) and electrochemical porous etching. For wafer-sized area fabrication, interference lithography and deep reactive ion etching (DRIE) are combined [32]. The master stamp on silicon or silicon dioxide is usually prepared using one of the above-mentioned lithography processes and then etched into the substrate material through reactive ion etching.
As the number of steps increases, the complexity also increases, which may not give the desired outcome. In order to minimize the steps involved in making the masters, resist-based molds were made and tested using SU8 and HSQ resist in photolithography and e-beam nanolithography based stamp making respectively [20]. The existing micro/nano-patterning techniques to study cell behavior have their limitations: they are expensive, time-consuming and only capable of making small sample sizes because the existing processes are generally for making smaller devices in the semiconductor industry. In order to produce many samples, soft lithography processes such as nano-imprinting and hot embossing techniques are used.

2.2.2 Polymer Microfabrication

Polymer-based fabrication processes are used in replicating structures defined on a hard master into a soft material such as polymers or elastomers. These microfabrication methods on soft materials are low-cost alternatives to silicon or glass-based fabrication processes. These are also suitable for high-volume productions. Nano-imprinting, hot embossing and PDMS stamping are some of the main polymer-based lithography methods used for biological and microfluidic type of applications for replicating micro- and nanoscale features [33]. In PDMS stamping, the elastomer poly(dimethylsiloxane) is poured over the hard master, cured and then released. In hot embossing, patterned defined on a hard stamp is pressed onto the planar polymer substrate with a controlled pressure and temperature. Detailed hot embossing review is presented here, as this is a method extensively used and optimized in this work.
2.2.2.1 Hot embossing

The hot embossing process is typically used in transferring micrometer structures into polymer materials. The thin planar polymer sheet is positioned, heated above its glass transition temperature (Tg) and then compressed with an applied force using a hard stamp on the pressing plate as illustrated in Fig. 4 [34].

![Hot embossing process illustration](image)

Fig. 4: Hot embossing process illustration Elsevier. Reprinted with permission from © 2009 Elsevier [34]

After pressing with a controlled forced, the system is allowed to cool down while in contact and then the substrate is demolded from the stamp/master.

Thermoplastic materials are generally used in this process as polymer casting sheets. When heat is applied, a thermoplastic material becomes soft and then it returns to its solid state upon cooling. While being heated, the material goes through specific temperature regions and an important temperature point in the process is the glass transition temperature [36, 37, 38]. This is the temperature where the polymer material goes from its hard or brittle state to viscoelastic or rubbery state. Within the glass transition temperature region often given with a lower and upper limit, the amorphous polymer stays as viscoelastic state which is ideal for molding purposes because when it cools back down to below Tg it retains the shape of the mold and becomes sufficiently hard to be demolded and the material turns into a glassy state. The material that we
will be investigating is PMMA which is an amorphous thermoplastic polymer which has a Tg of \( \sim 105 \, ^\circ C \). In the amorphous state the polymer chains are irregular and they maintain irregularity even while heating and cooling the substrate as in Fig. 5 but the chains are elongated while heating [34]. Amorphous polymers have wide softening region and they show moderate resistance to heat, high resistance to impact, low shrinkage and optically transparent.

![Diagram of chain regularity change with heating and cooling](image)

Fig. 5: Amorphous, semi-crystalline and liquid crystalline thermoplastics and their change in chain regularity with respect to heating and cooling process

In crystalline polymers the upper limit is often considered as melting temperature, and the polymer chains start to melt and degrade but in amorphous polymers there is no clear transition in melting and therefore does not exhibit a specific melting temperature point. However in the thermal behavior of an amorphous polymer, different temperature ranges (that have no specific point) such as energy elastic range, glass transition range, entropy elastic range, flow range, melt and decomposition range are reported [34]. In the energy elastic range the polymer is in solid state and behaves like brittle or glass. In the entropy elastic range, the polymer behaves like rubber and it is ductile. The transition between glass and rubber state is defined by glass transition temperature which is the point of maximum change of enthalpy. Entropy elastic range is defined as the oriented macromolecules (characterized by lower entropy) tendency to go back to the initial, not oriented, state. When the temperature is continuously increased the polymer
goes into a flow region which is defined as the range of temperature where the transition between rubbery state to melting state occurs continuously. Within this temperature range, physically bonded macromolecules become less effective and they glide off each other. Significant reduction in shear is noticed in this range. As temperature is further increased, the polymer gets into the melting range where the large temperature interval decreases the shear modulus further. Increasing the temperature more will result in decomposition where macromolecules will be damaged by the thermal energy.

When the temperature increases to melt range in an amorphous thermoplastic polymer, there is sufficient energy for bonds to rotate within a polymer chain that result in lower modulus compared to crystalline materials. This will allow larger elongation of chains under a smaller load which makes an amorphous polymer ideal for hot embossing [34]. Since the shear modulus change happens over larger range in amorphous polymers there is a larger embossing window compared to semicrystalline polymers. This is because the decrease in shear modulus is very abrupt in semicrystalline polymers. At the beginning of the small window in semicrystalline polymer, the polymer chains are stiffer making it hard to emboss micro structures.

While using amorphous polymer like PMMA, there has been an upper limit to the embossing temperature noted and many studies seemed to have used up to 150 °C – 160 °C as their maximum emboss-temperature [40, 41, 50]. According to Kakumani, A, while embossing PMMA at around 160°C the stress relaxation curve relatively crosses the zero barrier and goes into the negative stress region when compared to curves at lower temperatures [40]. Therefore to have an embossed material with non-degradable quality it can be suggested that this is the upper limit for embossing PMMA.

The fields of microelectronics and microfluidics have utilized the hot embossing processes using various industrial embossing machines and lab-made machines [41]. N.S. Ong et al. embossed ~65 µm diameter and ~5 µm deep microlenses on polycarbonate (PC) substrates using a Si mold. In this paper the primary embossing temperature and force were investigated. Their findings suggest that the high temperature for the PC substrate was not desirable and that a balance between viscosity and the change in temperature while cooling had to be achieved. The surface quality deteriorated for the Si mold for higher embossing forces. For their application of the embossed microlens structures, a roughness (Ra) of 11 nm was the best surface finish and this
was achievable with 6.6 kN to 11.12 kN of embossing force [42]. In their study the focus was mainly on embossing force and temperature, but another main factor that can influence the embossing result - the embossing wait time - was not studied. Because of the difference in material properties, the result here can be useful for PC embossing but may not give the same outcome on PMMA substrates.

In another study, microfluidic chips for capillary electrophoresis applications were made with hot embossing processes using PMMA and PC materials[43]. A hot embossing temperature of 130 °C for 4 min emboss-time and 150 °C for 5 min emboss-time were applied for making channels with cross-sections of 100 µm by 40 µm on PMMA and PC, respectively. However, the master stamp here was made out of nickel and a very large embossing force was applied, which in the case of silicon stamp could damage the master. Therefore these specific parameters may be suitable for a hard master like nickel and may not work for silicon. In a study by Devendra K.M. et al., a silicon master was used at 120°C embossing temperature, a lower force 1.71 kN and a hold time of 10 min but only 30 µm of depth from a 50 µm deep silicon stamp was achieved [44]. Therefore, in order to achieve the same replica of the mold, the embossing parameters had to be optimized which will become specific to the material being used.

Nano imprinting is a method similar to embossing but in this case a thin residual layer of polymeric resist material is dispensed on the surface of the substrate, which is often a semiconductor substrate like silicon. This resist acts as a soft cushion between the hard mold and the substrate when pressed under a controlled pressure and temperature or cured under UV light, as illustrated in Fig. 6 [45, 47].

Fig. 6: Nanoimprinting procedure starting with spin coating of resist polymer, embossing, de-moulding and then etching to transfer pattern. Reprinted with permission from © 2007 WILEY-VCH Verlag GmbH & Co. KGaA, Weinheim [45].
This method is suitable for transferring nanometer-size features and it is followed by reactive ion etching to get an opening on the substrate. Chou et al. demonstrated nano imprinting with temperatures between 140°C and 180°C and pressure from 600 psi to 1900 psi by fabricating 75 nm deep patterns using PMMA resist on silicon substrates [46].

L.J. Heyderman et al. studied the flow behavior of PMMA while imprinting the resist-coated features by varying the viscosity of PMMA [47]. The dimensions of the features tested in this study ranged from nm to µm but the depth of the features were 70 nm and 175 nm. By varying the molecular weight (25, 75, and 350 kg/mol) of the PMMA, the viscosity of the resist was changed and then spin coated onto a silicon substrate followed by embossing/imprinting the master, which is silicon based hard stamp, into this substrate. From this study the researchers concluded that temperatures greater than 100 °C and 300 - 3000 Pa-s PMMA viscosities result in faster embossing times to completely fill the cavities. This study is mainly suitable for imprinting PMMA resins, as they did not use PMMA sheets and for nanometer depths. 10 - 40 kN of force were used, which is in the high range, but this did not affect the master most likely because the resin acts as a soft layer inhibiting crack formation.

Hot embossing was effectively utilized to transfer micro-structured V-groove surface from Si stamp on polyimide thermoplastic to study osteoblast cell shape and alignment by J.L Charest et al [60]. The V-grooves in this study were 5 µm deep, 4 µm wide with 34 µm period. They have reported with this specific design that the cell nuclei strongly aligned to the microgrooves but they did not elongate due to the micro structure.

Some parametric analysis of hot embossing done with different embossing tools has reported that for better replication results parametric optimization would be necessary [40,41, 50]. In these studies temperature, force, emboss time, and cooling times are studied for various embossing tools as these parameters are affected by many factors related to the embosser and materials being used. In one such parametric analysis, Chen-Hsien Wu et al. suggest height of features increased with embossing time and decreased with demolding temperature [41]. Although high embossing forces were required in this study, force did not influence the quality of the results. Demolding temperature was another factor that was extensively studied and the suggested range is between 60-85 °C to have a fracture free demolding [41, 62, 63]. From these researches that had been carried out so far in hot embossing, it was observed that other than demolding
temperature, the three main areas that played an important role in replicating a stamp were embossing temperature, applied force and wait time or emboss time. These three factors can depend on feature size, mold material and the machine. Therefore these could be tested for Jenoptik HEX 02 embosser as a detail study replicating features on Si based master onto PMMA substrates.

2.3 Characterization

Micro-structured surfaces are characterized with optical microscopes, which have 2D and 3D capabilities. Further analysis and nanoscopic characterization are usually done with scanning electron microscopy (SEM) on conductive surfaces and environmental SEM (ESEM) on polymer substrates. In order to analyze depth profiles and roughness of surfaces, atomic force microscopy (AFM) and profilometry measurements are carried out. Roughness measurements can tell accurately on how the depth varies across the surfaces.

Roughness

Roughness is defined as irregularities on a surface. When a stylus of a profilometer or an AFM plots the primary profile of a surface, the peaks and valleys in the profile can be evaluated for roughness characteristic of the surface. There are a number of parameters to quantify roughness profiles of the surface being analyzed. Roughness parameters are categorized into the following three groups: amplitude parameters, spacing parameters and hybrid parameters [48]. Among the three groups, amplitude parameters are more commonly used in science and engineering to measure the vertical characteristics of surfaces.

The following amplitude roughness parameters are more useful in studying surface topographies [48]. Within the amplitude roughness parameters, maximum Height of Profile (Rt), peak to Valley Height (Rz), arithmetic average height, (Ra) and Root Mean Square Roughness, Rq are four different measurements to characterize roughness of a profile. As two main arithmetic parameters used widely in industry, Ra and Rq are explained below.

**Arithmetic Average Height, Ra**

Arithmetic average height (Ra) is the average absolute deviation from the mean line within a sampling length (l).
\[ R_a = \frac{1}{l} \int_0^l |y(x)| dx \]  \hspace{1cm} \text{Equation 1 [48]}

This measurement is not sensitive to small changes in profiles but is easy to measure and is a universally known parameter to define roughness profiles for general quality control purposes.

**Root Mean Square Roughness, \( R_q \)**

\( R_q \) is the root mean square average of the absolute ordinate values within a sampling length. This measurement is more sensitive than the arithmetic average parameter (\( R_a \)) but it is more useful in describing roughness profile in terms of statistical methods.

\[ R_q = \sqrt{\frac{1}{l} \int_0^l \{y(x)\}^2 dx} \]  \hspace{1cm} \text{Equation 2 [48]}

In this project surfaces are characterized for their roughness profile in order to compare embossed surfaces with their masters. For a perfectly embossed substrate it is expected that the roughness profile to match with the master’s roughness profile. As \( R_q \) is more sensitive than \( R_a \), it is used in describing roughness profile in terms of statistical methods.
Chapter 3
Hot Embossing

3 Hot Embossing Study

In the following chapter the hot embossing process will be optimized with a view of producing larger areas of micro-structured surfaces. The hot embossing machine used in the study was a Jenoptik HEX 02, available on campus at the UHN Micro fabrication Center facility [51]. This embosser model has automation capabilities and is suitable for pilot production and research and development applications [52]. The embossing settings are usually tested each time based on one’s specific requirement such as feature size and materials. Here we try to generalize the process for making embossed PMMA surfaces with a silicon-based stamp/master.

3.1 Methodology

3.1.1 Mask Designs

For the studies on making embossed surfaces with the HEX 02 machine and to be able to use for algal biofilm studies, a number of different masks were designed. The first mask with micrometer lines (2 µm lines spaced at different distances) and micrometer lattices (2 µm diameter) arranged in square pattern (with different distances) was designed using L-edit software. These particular patterns were chosen to examine if features as small as 2 µm can be fabricated with the embosser and to fabricate many different features at once on one substrate in the meantime to test if lines and square lattices had any effects in algal biofilm formation. Each pattern was about 5 mm x 5 mm (Fig. 7). The design was then sent to Nanofab University of Alberta and was printed as a Chrome-Glass mask.
Fig. 7. A schematic representation of the layout of square lattices and line patterns with diameter 2µm and width of 2µm spaced at 5 – 85 µm distance on Mask 1

The second and third masks with only vertical and horizontal lines were designed with the use of L-edit software. These masks designs were chosen after observing very less effect with algal biofilm on the smaller features fabricated from Mask 1. The area of patterns was too small to be tested for algal productivity. In this case the patterns needed to be on a considerably larger area but had to be practical from the fabrication standpoint. This is because current fabrication facilities have tools that are capable of handling 4” wafers or 5” masks. Therefore the features were made on an 8 cm x 2 cm area that is enough for testing of algal biofilm and can be fabricated with a 4” Si wafer. The features were selected as lines because they are easy to make with lithography and in most early cell related studies on topographical cues, line features are first to be studied as the structures a long and repeated. The mask with lines that went longitudinally (8cm long) was called the vertical line mask (Mask 2) and the mask with lines going horizontally (2cm long) was called the horizontal line mask (Mask 3). These lines were each 10 µm wide and separated by 30 µm (Fig.8).
3.1.2 Stamp Fabrication

The stamp was fabricated with Mask 1 (Fig. 7) for preliminary studies of hot embossing and algal biofilm tests. A new 4" Si wafer was first cleaned with acetone sonication for 3 min, next an IPA (isopropyl alcohol) rinse for 1 min duration was used to remove the acetone. This was followed by de-ionized water rinse for 1 min and then the wafer was blow dried with nitrogen. The sample was then subjected to a dehydration bake was then followed for 2 minutes at a temperature of 105 °C. After cooling down the wafer, adhesive primer hexamethyldisilazane (P20-HMDS) was spin coated on the wafer at 4000 rpm for 40s followed by a positive resist coating (MICROPOSIT® S1811® PHOTO RESIST) at the same speed and time. The resist-coated wafer substrate was soft-baked to remove solvents at a temperature of 105 °C on a hotplate for 2 min.

The chrome mask was placed on the mask aligner (Suss MicroTec MA6 Mask Aligner) followed by loading the resist-coated wafer into the machine. The machine was then enabled to expose the wafer under UV (365 nm wavelength, 16.9 mW/cm² intensity) in hard contact mode for 10 s. The wafer was then unloaded from the mask aligner and developed in MF-321 (MICROPOSIT® MF®-321DEVELOPER) with gentle agitation for 40-50 sec or until the resist cleared out in the exposed areas followed by DI water rinse for about the same time. The wafer was inspected under an optical microscope to ensure that the resist had been removed from all the exposed areas of the substrate. The substrate was hard-baked at 105 °C on a hotplate for 2 min in order to harden the resist to improve adhesion and thereby etching of the wafer.

Dry etching was carried out using an inductively coupled plasma reactive ion etcher (Trion Phantom II RIE/ICP System). The recipe for etching at a rate of approximately 400nm/min included SF₆, O₂, CHF₃ and He etchants with flow rates of 30 sccm, 20 sccm, 12 sccm and 10 sccm respectively, pressure of 100 mTorr and RIE RF power of 120 W.

Fig. 8: A schematic representation of line masks. The lines were 10 µm wide each line is spaced at 30 µm
3.1.3 Hot-embossing

The hot embosser Jenoptik HEX 02 (Fig. 9) was used for transferring patterns from a hard stamp to a polymer.

![Jenoptik HEX 02 Hot Embosser](image)

Fig. 9: Jenoptik HEX 02 Hot Embosser

There is a standard recipe or macro function code which comes with the machine to control the embossing process such as the one given in Fig. 10 and the control functions are explained in the manual [64]. The parameters such as force, temperature, time, velocity and demolding can be changed based on the different embossing needs. The process commands start with closing the door and initializing the force unit. As a next step, the heating is enabled for the Top and the Bottom plates of the embosser while cooling control is off. The temperature can range from 20 to 220 °C. It is recommended that the heating temperature be set higher than the operating emboss temperature to get the heating process going. The vacuum chamber is then closed and the evacuation is triggered.
The tool and substrate move towards each other and come into contact with the next command. Both plates move at a defined velocity up to the defined position. The motion of the punch is stopped when the maximum set force has been reached during this positioning step. The chart window which shows the temperature, position and force controls in real-time will appear on the computer screen as in Fig. 11.

![Chart Window](image)

**Fig. 10:** A Typical macro command of the embosser

![Real time process monitoring chart](image)

**Fig. 11:** Real time process monitoring chart from HEX 02 displaying time, force, position and temperature
A minimum touch force is applied. This is applied in order to facilitate heat conduction before the actual heating step. As a next step the function waits until a target temperature has been reached at a particular channel/sensor or at all sensors. The temperature sensors are located for the top plate, bottom plate, tool and substrate. The channel number indicates the sensor number at which the target temperature has been monitored before it moves to the next command line [64]. This target temperature is set just above the embossing temperature so that the heating is still enabled. Heating command is then enabled in the next step to keep the top and bottom plates at the embossing temperature. The embossing temperature has to be set at a temperature above the glass transition temperature of the polymer being embossed.

Force is then applied in the next command line. The target force may vary from 100N to 200kN. Velocity may also be varied between 0.0001 to 600 mm/min [64]. While the target force is being applied the unit can wait for several seconds with a ‘wait time’ command line. This can be set between 0.05 to 90000 seconds. As a next step, the cooling is enabled for top and bottom plates. This switches off the hot plates. The temperature is monitored until it reaches a targeted cooling temperature at a particular channel or all channels/sensors and moves to the next line. The next command is another wait time, which allows the substrate features to cool down while being in contact with the stamp. The demolding function is enabled and this allows the work piece to be separated from the mold automatically. The vacuum chamber is then vented and opened, and after this the substrate is extracted. This is the general operating procedure outlined in the hot embosser manual and this order of the function is maintained but parameters are chosen for particular embossing needs [64]. For example, for different polymers the embossing temperature, force and wait time has to be optimized because these parameters can depend on the material parameters such as glass transition temperature and viscosity etc. Similarly, a higher force can damage a fragile stamp whereas a very strong metal master can withstand high embossing forces. Therefore in order to understand the different factors of this embossing machine and the nature of embossing needs, qualitative embossing runs or preliminary embossing runs were initially carried out.

The standard recipe by Jenoptik for PMMA embossing, recommends a high initial heating temperature and a high force of 20kN. Emboss runs with similar recipe was followed in the preliminary studies. Preliminary embossing runs were done with the use of stamps made out of Mask 1 and the resulting embossed features were checked under optical and electrical
microscopes. A range of standard forces (5kN, 10kN, 15kN, 20kN) and a range of embossing temperatures (120, 130, 145 °C) which are above PMMA’s glass transition temperature as used in other similar studies and recommended in embossing procedures described by Jenoptik’s standard PMMA embossing and Ualberta nanofab center were tested [53, 54]. A small wait time of 60s to a larger wait time of 600 s were used in these studies.

The importance of the vacuum chuck for top plate, using glass on the bottom plate, different forces and wait time that can be used for a Si master, the cracking behavior of the Si master and demolding effects were qualitatively tested in these preliminary runs. No coating was applied in between the stamp and substrate in the initial experiments. However, after several breakages of Si masters as explained in Section 3.2.1, silane ((tridecafluoro-1,1,2,2-tetrahydrooctyl)-1-trichlorosilane, from UnitedChem) was dispensed along the wafer’s edges and left under the fume hood for roughly 24 hours before the embossing was carried out.

After the preliminary tests it was obvious that force, temperature and wait time played important roles in providing good quality embossed materials. Therefore in order to systematically study the effects of these three main factors of embossing using the Jenoptik HEX 02 machine for making particular feature size, silane-coated wafers with V grooves were used as stamps. The parametric analysis was carried out in the following fashion by changing one parameter at a time as illustrated on Table 1. As in the preliminary experiments, emboss material used here was PMMA (1.5 mm thickness supplied by Plastic World).

Table 1. Parametric analysis (Force, Temperature and wait time tests) of hot embossing using Jenoptik HEX 02

<table>
<thead>
<tr>
<th>Tests</th>
<th>Force (kN)</th>
<th>Emboss Temperature (°C)</th>
<th>Emboss Hold Time (s)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Force Test</td>
<td>1</td>
<td>130</td>
<td>100</td>
</tr>
<tr>
<td></td>
<td>5</td>
<td>130</td>
<td>100</td>
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<td></td>
<td>9</td>
<td>130</td>
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<tr>
<td></td>
<td>13</td>
<td>130</td>
<td>100</td>
</tr>
<tr>
<td>Temperature Test</td>
<td>5</td>
<td>115</td>
<td>100</td>
</tr>
<tr>
<td></td>
<td>5</td>
<td>130</td>
<td>100</td>
</tr>
</tbody>
</table>
The stamps for these studies were fabricated in the same way as deep V-grooved micro-structured surfaces were fabricated for algal biofilm growth explained in Section 4.1.1. The V-grooved Si based stamp was then treated with silane and left under the fume hood for 24 hours. Then the master was embossed and tested for its effects on the force while keeping the temperature and wait time constant. A range of forces (1kN, 5kN, 9kN and 13kN) was tested in this study. From the initial studies it was noticeable that the Si stamps were susceptible to breakage at very large forces. Forces around 20kN recommended for similar embossers with Si mold [54]. However, in the initial studies, even 10kN was too much for these Si stamps. It also could depend on feature sizes as explained in Section 3.2.1. Therefore the embossing force will depend on the particular need and the feature size. Thus it is ideal to keep the force as small as possible to efficiently use the silicon master for many repeated replication process. In this force test how small the force can be is what being tested here while keeping all other factors identical for each run.

In the second study, the emboss temperatures were tested while keeping the force and emboss wait time constant. It has been recommended that the embossing temperature be kept above Tg of the polymer being embossed. The glass transition temperature for PMMA is 105°C but there is
an upper limit for PMMA embossing reported as 160 °C and many studies have used temperature below 160 °C which is also in the melt range of PMMA [40,50]. Also amorphous thermoplastic has a wide softening region and since this is a large range, in order to determine the best embossing temperature three temperatures between the lower and upper temperature limits were tested using the Si stamp. The three temperatures tested in this study were 115 °C, 130 °C and 145 °C.

As a third study, the wait time was tested while keeping the force and temperature constant. From the preliminary studies, a longer wait time was desired for achieving the same depth of the master. The time required to fill the entire depth of the stamp had to be systematically determined. Its dependency on the force was also interesting because the hold time command is enabled while force is still being applied to the system. A range of wait times from 30 s to 660 s was tested for a 5kN and then for a 10kN applied forces. The maximum of 660 s wait time was selected because more than 10 min for each run will commensurate to a total emboss run of 30 min or more, which is very inefficient if 10’s or 100’s of replicas were needed. For algal biofilm study many samples are required which would need to be embossed in very short period in order to be efficient. Therefore the goal for this test is to find the shortest time needed to transfer the patterns efficiently.

From the three parametric studies, it was obvious that time was one of the main factors along with applied force affecting the overall process in transferring patterns efficiently as discussed in section 3.2.2. Therefore as a final experiment, PMMA resist (which is liquid resin in room temperature) was used in addition to the PMMA sheet and tested under a lower emboss force (5kN) and lower wait time (100s) (Fig. 12). PMMA resist (950PMMA A3 (3% in Anisole), MicroChem) was poured and spread evenly on top of the PMMA sheet and was left under the fume hood for about 2 hours [68]. It formed an even layer and started solidifying with the PMMA sheet but was still viscous which is when it was embossed with a V-grooved Si stamp.
3.2 Results and Discussion

3.2.1 Preliminary Quality Analysis

Stamps fabricated following the procedure outlined in Section 3.1.2 were used in embossing PMMAs for quality check of embossed surfaces. The optical microscope was mainly used in understanding the resulting embossed surfaces and the setting of embossing procedure as a quick check. The embossing was carried out in the initial experiment using a sandwich method where Si stamp was placed directly on top of the PMMA substrate. While doing this the force was being directly applied to the Si stamp which crushed the wafer for most of the runs. When the top plate was equipped with a vacuum chuck, the wafer was placed on the top plate using the vacuum. Although it fell on top of the substrate during demolding, the force was not directly applied to the wafer when the top plate started approaching the substrate. This was important for a stamp that was as big as a wafer. For smaller stamps (Si wafer pieces), sandwich method was still satisfactory as the area of force being applied to was smaller therefore the impact was not as high as on a larger wafer.

While during the quality of embossing results the importance of the glass plate for a large patterned surface transfer was investigated. Although the glass plate was part of the machine but because of the brittle nature of glass under high embossing forces it had to be replaced very often. A glass plate was not used in some of the initial hot embossing processes and the PMMA sheet was placed directly on the bottom holder made out of metal. In this case some parts of the embossed surface became covered by liquid phase and non-uniformity was noticed across the whole surface as in Fig. 13, which becomes an issue when using a whole Si wafer as a stamp and not when a smaller piece of Si was used as a stamp.
For a whole wafer being used as a stamping master, a liquid phase was covering some parts of the features. This was not an effect of high emboss force or high temperature as this was observed for all forces and temperature settings. When a glass plate was placed on the bottom holder, the liquid phase covering disappeared. The glass plate acts as a uniform heat transfer plate. Although a metal plate (which is the real bottom holder) transfers heat better than a glass, it would do so in a quick manner, which for the most part will be non-uniform and undesirable for this purpose. In metals atomic vibration and free electrons participate in transferring the heat through a conduction process. In a non-conductive material such as glass there are no free electrons so only atoms conduct the heat, hence the process will be slower but the heat distribution will be uniform. Therefore it is important to have a glass plate for uniform heat transfer from the bottom holder to the embossing material so that when embossing is enabled throughout the PMMA material, there is a uniform temperature across the embossing surface. Especially when embossing large area of features using a whole wafer, the uniformity of heat across the polymer substrate becomes important.

Since a range of patterns was fabricated on a single master stamp with lines and lattices separated by different distances, there was a difference in which each pattern was demolded. At the end of the process, the machine demolding function did not completely demold the stamp from the substrate although the vacuum chuck was used in holding the stamp. The stamp fell onto the substrate most likely when the demolding command was enabled. The stamp and polymer had to be taken out of the machine and left to cool down outside on a wet-bench and
allowed to demold on its own. In some cases the sheets were manually pulled out of the master, causing defects in patterns as shown in Fig. 14.

![Fig. 14: PMMA substrate after manually peeling off the Si master](image1)

To avoid damaging the structures by manual demolding, the stamp and substrate were left to cool down for several more minutes (10-15 minutes). While some patterns were demolded easily on their own after more time was given to separate, some other patterns with features that are separated by smaller gaps were intact for several more minutes; eventually, at the attempt of demolding on its own, the Si master broke into pieces by popping out itself as in Fig. 15.

![Fig. 15. Si master breaks into pieces after leaving to demold on its own outside the embosser](image2)
This type of breaking of the Si master was repeatedly observed in the smallest line features made out of Mask 1 and in some substrates made out of Mask 2 in the later studies. Smallest line feature had 3 µm lines separated by 3 µm spacing and it was about 1 – 0.5 µm deep. Compared to the other patterns on the same wafer, this one was the narrowly spaced pattern with the smallest dimensions of all. This type of wafer cracking could possibly be happening through crack propagation in silicon <111> planes, which have the highest probability of fracture through cleavage compared to other planes in a silicon wafer. This could be due to the tension built up between the features due to the difference in thermal expansion/contraction coefficient of the two different materials in contact. The coefficient of thermal expansion for Si is $2.6 \times 10^{-6} \degree C^{-1}$ and for PMMA is $50-100 \times 10^{-6} \degree C^{-1}[55]$. Linear coefficient of thermal expansion is defined as the relative change in material length per degree temperature change. This means the PMMA is faster at contracting and expanding compared to silicon while cooling and heating respectively. However, when phase also changes with increasing or decreasing temperature, it becomes more complex. In silicon, since there is no phase change, it will be purely a linear thermal expansion. In PMMA however, there is a phase change from rubbery state to viscoelastic fluid and then it becomes hard material retaining the mold shape through the embossing cycle. Although it is assumed that while demolding the PMMA already has stabilized in one phase (below Tg), if there are some parts within the material that are still undergoing a phase change while cooling, then it may lead to microcracking and thermal expansion hysteresis [56]. Thermal stress due to the difference in thermal expansion could cause this type of fracturing in the stamp. When there is a difference in aspect ratio in features being embossed, the span of contraction will be different for PMMA along the width and the height of the features that will also impact the Si stamp. This can induce fracturing along the more susceptible plane of Si. Due to this cracking of Si, only one PMMA was being stamped per one stamp.

Since the two materials are being intact together is what causing the thermal stress between them and to avoid such cracking, some kind of anti-adhesive layer had to be coated between the two materials. A silane-based anti-adhesive coating is often used in PDMS demolding. A similar silane anti-adhesive coating was also reported to have been used in imprinting and embossing by research groups and laboratory procedures [57]. After applying (tridecafluoro-1,1,2,2-tetrahydrooctyl)-1-trichlorosilane anti-adhesive coating, the demolding of the stamp and substrate was much easier. By coating the silane the Si surface has become hydrophobic as
illustrated in contact angle measurements Fig. 16 (Goniometer). The V-grooved Si stamp had a contact angle of 43.44 ±0.406° before coating silane and the same stamp had a contact angle of 130.553 ± 1.528° after coating silane.

Fig. 16: Contact angle measurement on patterned Si a) without silane coating b) with silane coating

The silane coating did not affect the resulting PMMA as the contact angle measurements remained similar on those PMMA’s that were embossed using Si wafers with and without silane coating. The PMMA contact angles were 87.805 ± 0.242° and 85.793 ± 0.434° for embossing using Si without and with silane coating respectively (Fig. 17).

Fig. 17: Contact angle measurements on embossed PMMA a) replicated using Si that had no silane coating b) replicated using Si that had silane coating

Silane treatment definitely allowed break-free demolding of Si and PMMA. Silicon stamp breakage did not occur for several runs. However, it was noticeable that re-coating of the silane was needed after 5 consecutive embossing rounds.

In addition to coating silane, demolding temperature also played an important role in reducing thermal stress. It is ideal that the temperature is kept constant around 60°C for a fracture-free demolding [62, 63]. The optimum demolding temperature was defined based on a combined effect of adhesion and friction between the two materials. In such cases after the champer opens up instead of taking the substrate and stamp out of the embosser they can be left inside the hot embosser’s bottom plate at 60°C and let it demold on its own.
It was also very obvious that as embossing force increased, the master Si stamp was susceptible to breakage and crushing as in Fig. 18. When the Si wafer was crushed like this, it was impossible to use the stamp for another embossing round.

Fig. 18: Si master embedded and crushed into the PMMA making it impossible to demold and reuse of the master

This crushing effect was observed to be the result of high force being applied on the Si wafer. While demolding, the machine applies even more pressure to the surface than the applied force for embossing. If the emboss force was 5 kN, while demolding the machine presses the surface to pull apart with a force as high as 10 kN. Similarly if 10kN force was used for embossing, the machine applies up to 15 kN force to pull apart the top and bottom plates. This has been reported on the manual of HEX 02 to be the case while demolding. The chamber will be compressed when evacuation happens. However in addition to that in almost all the emboss runs, the compress air switches on which pops up as an error message allowing the user to retry the demolding process. This could be the reason why high forces were being pressed on to the substrate while demolding and in some runs causing damages to the Si stamp as in Fig. 18. According to the manual while demolding, the demolding ring has to completely press onto the substrate otherwise an error message will pop up. It is therefore the suspicion that when the vacuum chuck was installed to hold the wafer on the top plate, the demolding ring could have been blocked. This causes compress air being forced on to the substrate causing damages to Si mold while demolding. Vacuum chuck is needed especially for embossing full wafers because if the sandwich method is used then the large emboss force will be applied directly to the Si wafer causing damages to it in the beginning of the process itself.
Some of the master surfaces were imaged using SEM. Similarly embossed PMMA sheets were imaged using SEM at low voltages (1kV and 0.5kV) as in Fig. 19.

![SEM images](image)

**Fig. 19:** SEM images of a) grooves on Si, b) square lattices on Si and, c) inverted trenches on PMMA and d) inverted square lattices on PMMA

These characterizations reveal the depth/height of the features achieved with the preliminary emboss settings of 5kN force, 140 °C emboss temperature and 120 s emboss wait time. It was noticeable that the PMMA only reached half of the depth defined on the master stamp (1 µm) in this particular embossing.

### 3.2.2 Hot embossing Parameter Tests

Throughout the preliminary hot embossing runs, it was observable that the force, temperature, and wait time were three of many factors that affected both the stamp and the substrate along with the efficiency of embossing number of replicas within a short period of time. Increasing the force had an impact on the Si master. Therefore knowing what is the minimum force to get a good quality replica was important. This needed to be studied in a more systematic way to
understand the quality of replicas being made with the chosen forces. Additionally, temperature for PMMA embossing had a wide range (105 to 160 °C) and knowing which temperature can result in good quality replicas is important. Furthermore wait time can affect how efficiently can a replication process be made in a very short period of time. After the embossing runs for parametric analysis were made, the embossed surfaces and the Si stamps were characterized using profilometry (Dektek 3) in order to quantify the quality of features. Using this the step height of the features on the resulting surfaces were compared with that of the Si stamp.

![Force test, at T = 130 °C and Wait time 100s](image)

**Fig. 20:** Resulting depth vs. applied forces at constant temperature and wait time, compared with the Si master's depth

During the force test, the temperature and wait time were kept constant at 130 °C and 100 s and four different forces were applied for four embossing runs using the same stamp. The depths of the resulting embossed patterns fabricated with each force were characterized under profilometer at different spots along the substrate surface and the depth values were averaged. On Fig. 20 the average depths of the embossed PMMA features at different forces were plotted. Also the actual depth of the patterns defined on silicon stamp was also drawn (orange) to compare embossed depths. This is the actual depth that the replicated features on PMMA have to reach if it replicated the master consistently. To attain the same depth profile as the silicon stamp (completely filling the cavity) of ~18µm, at the chosen temperature and wait time, the highest force of 13kN was required. This was definitely a large force that has the potential of damaging...
the stamp. When more replication processes are carried at this force repeatedly the mold will degrade at a faster rate. Even after using anti-adhesive coating, the stamp will only withstand a limited number of embossing runs because very large tension is being imposed on the stamp at this large force, which can degrade the stamp usage. It is therefore ideal that the force is chosen somewhere below 10kN so that more replicas can be reliably made with one stamp having anti-adhesive coating. It was also noticeable anti-adhesive coating was required after every 5 or 6 consecutive embossing runs.

The temperature was the other parameter that was studied. Three different temperatures were chosen above Tg of PMMA and the upper limit (160 ºC), while keeping the wait time and force constant. The upper limit was attributed to the stress relaxation of PMMA where it has been reported to be crossing the negative stress realm at this temperature and a constant strain. For embossing it is ideal that the stress is kept positive meaning that the chains are elongated to so better embossing results can be attained. For a constant 5kN force and 100s wait time, all three temperatures were resulting in statistically similar depths (~13 µm) but the depths are way below the actual size of the stamp groove (Si groove depth ~17 µm) (Fig. 21) in this study.

Fig. 21: Resulting depths vs emboss temperature at 5kN and 10kN applied force, compared with the Si master’s depth.
While keeping the wait time at 100s but increasing the force to 10 kN, the temperature test resulted at a more consistent and similar depth results as the stamp. It again showed no variation between the three temperature points considered. Flow of PMMA was not affected much by changing the temperature within the specified temperature region, but force played an important role in enhancing the flow inside the features. Although 10kN gave the optimal results in terms of depth and matched with the stamp that was used for this particular study, at 10kN the stamp tends to break as observed in many preliminary studies. Therefore achieving the replica at lower than 10kN force was required. Since there was no change between the three temperatures tested in this study, more temperature points close to the upper limit should be tested in the future to see if those temperatures will bring the viscosity down and enable flow at smaller forces. Considering that the chosen temperatures did not play much of a role in the embossing result or the quality of the replica, it is now time to check the emboss wait time dependency.

The time both the master and substrate are in contact while the force is still being applied is termed here as the wait time or emboss hold time. This wait time is important to be tested because in the quality tests, longer wait time was preferred because it gave the PMMA enough time to reach the grooved patterns but it was unclear what would be the best wait time that is efficient and not time-consuming for a chosen, temperature and force. This test was done at a constant temperature of 130 ºC and 5kN force and using the same stamp the study was repeated at a constant 130 ºC temperature and 10kN force. The emboss wait time started at 30s and went up to 660s, and it is noticeable (Fig. 22 below) that a longer wait time was better when a 5kN force was applied because at a lower force, viscoelastic PMMA flows into the grooves at a very slow rate and reached close to the depth of the Si stamp (orange line) only at the maximum time tested at this force. As for 10kN force, it reaches the groove depth at 240 s wait time and it goes beyond the actual depth of Si. It was slightly above the actual depth because while demolding, the features could have got elongated when there is an abundance of viscoelastic fluid at this force, temperature and long hold time setting.
The points are connected just to give guidance to the eye. To conclude about trends, more points will have to be plotted and compared to models. However it is reliable to say in order to get the specific feature depth the wait time had to be increased at smaller forces. Higher force of 10kN can replicate ~20 µm features within 4min holdtime but at this force the Si stamp can be damaged over repeated embossing. Also in general, it takes a total of 10-15 minutes to complete one emboss run. This total time was needed in order to do the general stamping procedures such as regular temperature commands and force activation and de-molding with a small wait time. Therefore if the smaller force as close to 5kN is used to overcome the stamp damage in the long run, a longest emboss wait time will be required, then it would take more than 20 minutes to complete one run.
In the real-time process chart (Fig. 23) for a 5kN emboss run with a shortest hold time of 30s, the temperature (red steep line starting at 136.5 °C) drops down to start the cooling process within a very short time right after the emboss force reached the set force of 5kN (the instant at which the blue line reaches 5kN), showing the stamp and substrate was only in contact for a very short period of time at the emboss temperature. On the other hand, when the hold time was increased upto 310 s (Fig. 24), the stamp and substrate were in contact for a longer period while the force was being applied. During this longer wait time, the polymer has enough time to reach its viscoelastic state and then for the rest of the time it flows into the features of the stamp.

Fig. 23: Real-time process chart for 5kN emboss force and 30s wait time embossing
From these studies although a larger force gives the desired depth in the polymer, a smaller force is ideal to preserve the stamp for many replication but it can be time consuming since a larger emboss wait time would be required to get viscoelasticity of the polymer which allows it to get the deep grooves of the master. It is noticeable that flow of PMMA is important and its flow time plays a crucial role when it comes to using a smaller force and smaller hold time. With the smaller force, it is not being pressed enough for the PMMA to flow faster into the grooves. In order to find solution to this issue, PMMA e-beam resist in addition to the PMMA sheet was embossed together. The PMMA resist is already less viscous at room temperature so when both PMMA resist and sheet are combined together, the viscous state is achieved in less time. Therefore the flow will be faster and with a less wait time and less force the embossing could result in the desired depths. Imprinting technology has used liquid form of the PMMA often but it was spin coated on a hard substrate like silicon and then imprinted and etched into silicon for different purposes. Here since PMMA has to be the embossing material (algal biofilm growth needs a transparent, biocompatible material like PMMA) and since both are the same material but in different forms there is a chance of using this e-beam resist together with the embossing sheet to achieve faster flow into the grooves. It is predicted that it will enhance the flow of PMMA even at a lower force and as it is already in liquid form the embossing can be done in a very short time.
Fig. 25: Depths of features embossed onto PMMA sheet, PMMA resist and sheet compared with the Si master

Table 2: Emboss parameters used for PMMA sheet and PMMA resist+sheet embossing along with resulting height measurement for comparison

<table>
<thead>
<tr>
<th></th>
<th>Force</th>
<th>Temperature</th>
<th>Wait time</th>
<th>Height/Depth(µm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Si stamp</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>17.55±0.70</td>
</tr>
<tr>
<td>Embossing with</td>
<td>5 kN</td>
<td>130° C</td>
<td>100 s</td>
<td>9.66±0.59</td>
</tr>
<tr>
<td>PMMA sheet</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Embossing with</td>
<td>5 kN</td>
<td>130° C</td>
<td>100 s</td>
<td>18.27±0.25</td>
</tr>
<tr>
<td>PMMA resist on</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>PMMA sheet</td>
<td></td>
<td></td>
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<td></td>
</tr>
</tbody>
</table>

This was tested at a 130 °C temperature, a less wait time of 100s and a smaller force of 5kN. The resulting depth from this test was compared with 5kN test at the above temperature and wait time on PMMA sheet alone and the silicon stamp used for this study (Fig. 25). The setting used and the resulting depths are listed on Table 2 as well. As predicted, the 5kN test with a small hold time of 100s on PMMA sheet (blue bar) resulted in very shallow depths and did not reach the
depth of the silicon (orange bar), however the resist coated PMMA (red) reached the depth at a lower wait time (100s) and a lower force (5kN). The PMMA resist was poured directly onto the PMMA sheet and left for about 2 hours to allow some adhesion to the sheet. It was still viscous when it was loaded into the embosser. Therefore the flow was enabled faster at a smaller force and shorter wait time. It is however not sure whether the PMMA resist was permanently adhesive to PMMA sheet or if special chemical treatment is needed in order to create some bonding between them.
Hydrophobicity of a material can be modified through micro-patterning and these surfaces can be made with conventional semiconductor fabrication processes. This can allow us to study algal biofilm’s interaction on a range of hydrophobic surfaces. Micro patterning increases the surface-to-volume ratio providing more surface area for attachment thus expected to result in a higher productivity of biomass. Irving, T had concluded in his preliminary study that micro-patterning on glass has a minor effect in the attachment of biofilms but had noticed that altering the hydrophobicity and increasing the surface-to-volume ratio may influence the initial algal biofilm attachment and an increased total biofilm mass [6,17]. Therefore the knowledge of how the algae and surface micro patterning interact is important in order to design and optimize a biofilm based photo bioreactor.

In this study semiconductor fabrication processes such as lithography and etching along with hot embossing methods were used in fabricating consistent micro-patterns in poly (methyl methacrylate) (PMMA) substrate in order to grow algal biofilms inside a photo bioreactor. The patterned PMMA coupons were added in the bioreactor and tested for the algal biomass productivity by collaborating researchers. The coupons were then inspected for algal growth on ESEM. Most of the work in this chapter has been published as a conference proceeding in Proc. SPIE 8923, Micro/Nano Materials, Devices, and Systems in December 2013 and has been included here with permission (Available online as Sathananthan, S. et al. doi:10.1117/12.2033794) [39].

4.1 Design and Fabrication

The coupons with micro-patterns were fabricated utilizing photolithography and hot embossing procedures. As a first step, Mask 2 and Mask 3 defined in Section 3.1.1 were designed using layout-editor software (L-edit) from Tanner EDA. The patterns consisted of lines that are 10 µm wide and spaced at 30 µm on an area of 2 x 8 cm² (Section 3.1.1). On Mask 2, the lines were 8 cm long and called a vertical line mask and on the other mask (Mask 3) the lines were 2 cm long.
and called a horizontal line mask. The patterns were printed on film photo plot masks from the Photoplot Store.

Shallow and deep grooves with inverted ‘V’ shapes on PMMA sheets were chosen to be tested for algal biofilm growth because preliminary experiments in algal productivity showed preferential algal growth along the edges of various material coupons tested. Therefore we decided to use the masks to make long edges with V-grooves [Scott Genin, Thesis work]. Shallow grooves were fabricated using SU8 resist on a silicon (Si) based master using both vertical and horizontal line masks (Mask 2 and 3) following photolithography process. Deep V-grooves were fabricated with the use of photolithography and widely used micromachining/wet-etching process of silicon dioxide wafer with the vertical line mask alone (Mask 2). Wet etching was chosen as it can selectively etch the planes of a Si wafer when it is masked with an oxide or nitride. While {100} planes are etched, {111} planes act as etch stop.

![Fig. 26. Schematic illustration of silicon plane and wet etching of Si](image)

The angle between {100} and {111} planes is 54.7° (Fig. 26). Therefore for a defined Mask 2 with lines that are separated by 30 µm spacing, the etch depth it can reach is, \(\tan 54.7° = z/15\) which results in a maximum of ~21.18 µm depth.

Hot embossing using Jenoptik HEX 02 embosser was to be used to transfer all three patterns into PMMA polymer sheets. Profilometry (KLA-Tencor P16+), roughness measurements and ESEM (QUANTA FEG 250 ESEM) were done on the masters and polymer surfaces.

### 4.1.1 Deep V-Groove Si Stamp

Deep V-groove stamps were fabricated with thermally oxidized Si <100> wafers. The oxide layer thickness was around 200 nm. The oxide thickness was measured with NanoSpec (Nanometrics NanoSpec/AFT 4000 Reflectometer). In order to improve adhesion of the resist to the Si wafer, an adhesive primer hexamethyldisilazane (P20-HMDS from Shin-etsu) was spin-
coated on the wafer at 4000 rpm for 40s followed by spinning of positive resist (MICROPOSIT® S1818® PHOTO RESIST, Shipley) at 4000 rpm and 40 s. These parameters were chosen according to the resist description and curves presented on the resist information sheet according to the thickness that needs to be achieved [65]. In order to remove solvents from the substrate, the resist-coated wafer substrate was soft-baked at 115 °C on a hotplate for 2 min in the initial experiments but after noticing resist being left on the substrate exposed areas, the soft bake temperature was reduced to 105 °C.

In the preliminary studies the resist-coated substrate was exposed with a UV Exposure Box system (Isel Vakuum-UV-Belichtungsgerät 1 UV Exposure Box) with a 1.25 mW/cm² intensity for 55s. The vertical line mask (Mask 2 described in Section 3.1.1) was aligned on the wafer with lines going parallel to the primary flat of the Si wafer and this aligns the lines on the mask with the silicon’s <100> planes. In the later studies however for a better and consistent exposure the resist-coated wafer substrate was exposed with the Suss mask aligner (Suss MicroTec MA6 Mask Aligner). The machine was enabled to expose the wafer under UV in hard contact mode for 10 s with the use of a quartz plate. The machine had a 365 nm wavelength and 16.9 mW/cm² intensity UV lamp. As a next step the wafer was developed in MF-321 developer (MICROPOSIT® MF®-321DEVELOPER, Shipley) with gentle agitation. The time of development was tested with series of repeated process and inspection under optical microscope. The best result of completing removing the resist in the exposed area of the positive resist was achievable at a development time of 40-50 s or until the resist cleared out in the exposed areas. This process was immediately followed by DI water rinse for about the same amount of time (40-50 sec). In order to improve adhesion and the etching of the wafer, the substrate was hard-baked at 115 °C on a hotplate for 3 min. Since positive resist was used in this patterning exposure and development steps cleared the exposed resist and left an opening on the oxide layer which can then be etched in the next step as depicted on the process diagram (Fig. 27).
Wet etching was carried out in the next step to completely open up the oxide opening which had resist on either side to protect the side wall features. The wafer was submerged into a buffered oxide etch (BOE, 10:1) solution for about 10 minutes and followed with DI (de-ionized) water rinsing. How long the BOE etch had to be carried out was ensured by inspecting the wafer in NanoSpec because different oxide thickness takes different etching time to be etched away. Therefore to ensure etch time based on the different oxide thickness on Si wafers in the initial experiments, NanoSpec was used to inspect if oxide has been completely etched away within the features. Usually for a 10:1 BOE solution the etch rate is reported as around 600 A/min however since BOE etching a selective etching it will only etch the oxide and will stop as it reaches the silicon [58]. While rinsing in water, the hydrophobicity of the water droplet can also be used to determine if oxide has been completely etched away in BOE solution. The oxide masking on a Si, completely wets the wafer however when Si has been exposed, the surface becomes hydrophobic and the water droplet beads off the surface. In the BOE etching process, the wafer was mounted in a Teflon holder and immersed into the solution carefully as BOE has HF concentration, the wafer was rinsed in water and blow dried with N₂ gun. After making sure the etching had completely cleared oxide within the slot opening of the resist acetone was used to remove the resist masking. As a final step, the wafer was wet-etched in a 45% KOH solution at a relatively stable 80±1 ºC temperature for a 17 - 20 μm deep etching. In this step, the KOH solution was heated inside a beaker, which was stirred at a constant rate. This was done on a thermometer-attached hotplate to be able to monitor and read off the temperature inside KOH solution. After the solution reached the set temperature and stabilized, the BOE etched wafer on a Teflon holder was immersed into the boiling solution. This etching process took up to 35 minutes at this setting. In some preliminary experiments, a 50% KOH was used at a stable 80
(±1) °C temperature and it resulted in a faster etch time (25 minutes) to etch 20 μm deep grooves whereas in the later experiments when only a 45% KOH was available in the lab it had resulted in a slower etch rate given that the temperature did not deviate much. In some reports for a 44% KOH solution, at 85 °C, the etch rate of 100 planes was reported to be 1.4 μm/min [29]. From the KOH etching of <100> plane it is reported that higher concentration of KOH can result in faster etch rates as long as the temperature is kept at constant [59].

In some earlier experiments the temperature fluctuated a lot (±5°C) therefore the etching was much slower. To ensure that the depth has been achieved with the KOH etching procedure, a 3D microscope was used (Nikon 3D Microscope) to inspect the 3D profile of the surface (Fig. 28). Features inspected in the middle of a KOH etching process was given on Fig. 28a, where the depth (8-10 μm) only reached half of what would have been achieved with the mask used in these fabrication. In this case the etching was repeated. On the second image a 3D profile is given for a Si that completely etched away the 100 planes and reached the actual height of the groove that would be achieved with the mask (~20 μm) (Fig. 28b).

This optical microscope is capable of giving 2D and 3D surface plot as in Fig. 28a) and b) for a quick check of the depth profile while etching in KOH solution. When this measurement was done in the microscope throughout the etching process, the wafer was taken out, thoroughly washed in running DI water and blow dried with N2. The etching was repeated until the desired height was achieved.
4.1.2 Shallow Groove Stamps

Shallow grooves were made with SU8 resist. For these SU8 based Si stamps, new silicon wafers were first cleaned with acetone sonication for 3 minutes, IPA (isopropyl alcohol) rinse for 1 minute and de-ionized water rinse for 1 minute and then the wafers were blow dried with nitrogen. Dehydration bake was then followed for 2 minutes at 100 °C. After the wafer had cooled down, a negative resist, SU8 3025 (MICROCHEM) was spun coated at a speed of 4000 rpm. The wafer was then prebaked at 65 °C for 2 min and at 95 °C for 15 min [66, 67]. UV Exposure was done in hard contact mode in the UV mask aligner (365nm, 16.9 mJ/cm²) for 22 sec. After the exposure the wafer was baked again on a hotplate at 65 °C for 2 min and at 95 °C for 15 min. Wafer was then immersed in SU8 developer (MicroChem) for 20 minutes with a magnetic stirrer inside. Hard bake was then followed for 2 hours on a hotplate [66]. The resist based silicon stamps, with lines going vertical and horizontal were made using the two masks designed (Mask 2 and Mask 3 described in Section 3.1.1) so that the substrate coupons would have lines that were perpendicular and parallel to the air diffuser inside the photobioreactor. Overall fabrication process to make shallow grooves is illustrated in Fig. 29.

4.1.3 Substrate Fabrication

Hot embossing method was utilized in transferring patterns from the Si stamps to PMMA substrates on which the algal biofilm was later grown. PMMA was used as a suitable material because the substrate is transparent, compatible to grow algae and is a good embossing material which is widely used for many applications.

Since the whole wafer was being embossed, the vacuum chuck was installed in the hot embosser’s top plate (Jenoptik HEX02) and the stamp was attached to this chuck and was held by vacuum. The substrate was placed on a glass plate to give a uniform heat transfer from the glass plate to PMMA sheet and held in place by two metal clips attached to the bottom plate.
After many embossing tests with different stamps the hot embossing parameters were optimized. However for this particular embossing for algal growth tests the substrates were embossed at a force of 10kN, at a temperature of 140 °C and were allowed to be in contact for 10 min after the force was applied. The cooling time was also 10 min.

<table>
<thead>
<tr>
<th>Feature Dimensions</th>
<th>Length of Groove</th>
</tr>
</thead>
<tbody>
<tr>
<td>Deep V groove PMMA</td>
<td>~30 µm wide, 20 µm deep, 40 µm pitch</td>
</tr>
<tr>
<td>Horizontal Shallow Groove PMMA</td>
<td>~4 µm wide, 1.5 µm deep, 40 µm pitch</td>
</tr>
<tr>
<td>Vertical Shallow Groove PMMA</td>
<td>~4 µm wide, 1.5 µm deep, 40 µm pitch</td>
</tr>
</tbody>
</table>

Approximate feature sizes on the fabricated coupons are listed on Table 3. Five deep V-grooved PMMA coupons, five horizontal lines shallow grooved PMMA coupons, five vertical line shallow grooved PMMA coupons and five smooth PMMA coupons were added into a Parallel Plate Air Lift (PPAL) reactor shown in (Fig. 30). This was designed by research collaborators to provide vertically grown algal biofilms with consistent lighting, nutrients, and shear [39].

![Reactors configuration and set-up](image-url)

Fig. 30: Reactor configuration and set-up [39]
4.2 Characterization

Stamps and substrates were characterized using profilometry to study the depth profiles of the surfaces. 2D profiles were taken on at least four different spots along the entire surface. On each profile, roughness of the flat part (between 2 V-grooves) and the slope part of the ‘V’ were separately measured as shown in Fig. 31. Root mean square (RMS) roughness (Rq) measurements were taken for each flat part and slope part of a profile from the master and the embossed PMMA to confirm feature size and consistency of patterns over the large area. The cross sectional ESEM imaging was also carried out on the master and the embossed substrate to understand the feature sizes.

Fig. 31: a) 2D profile of embossed PMMA. b) profile of flat surface c) profile of slope side d) Gaussian fit for roughness in the flat part e) Gaussian fit for roughness in the slope part [39]

Similarly for SU8 based masters, 2D profilometer measurements were completed. While fabricating these masters, exposure was too high for the SU8 resist which resulted in shallower
dips of 1-2 μm deep structures, each feature was 4 μm wide (Fig. 32). Although this was not an expected design, this was still included in the study to see if the feature size mattered. The reason was that the dips were shallower and had a tip like in deeper V-grooves structured surfaces. Therefore biofilm growth on the triangular edge like trench with different sizes can be compared.

4.3 Results and Discussion

The 2D profiles taken on the master were found to match with the inverted embossed PMMA substrates’ profiles. The profilometry roughness measurement results confirm that the patterns defined on the master stamp were consistently transferred onto polymer substrates using the hot embossing method. The RMS values on each flat part and the slope part were separately averaged and are presented in Fig. 33. Depth measurements are presented on Table 4.

![Fig. 33: Root mean square comparison on flat and slope parts of the deep V-grooved Si stamp and the inverted/embossed PMMA [39]](image)

<table>
<thead>
<tr>
<th>Substrate</th>
<th>Roughness (Rq) Flat portion (nm)</th>
<th>Roughness (Rq) Slope portion (nm)</th>
<th>Height or Depth (µm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Si stamp</td>
<td>1.48±0.66</td>
<td>11.56±2.46</td>
<td>17.77±1.28</td>
</tr>
<tr>
<td>Embossed PMMA</td>
<td>1.12±0.5</td>
<td>13.70±1.46</td>
<td>19.13±2.31</td>
</tr>
</tbody>
</table>
The flat surface is smoother than the slope section because the etching was mainly on the Si’s plane as opposed to the flat part, which was the oxide mask of the pattern. Considering the standard deviation of the roughness values, these results from the roughness measurements on flat and slope sections of the profile for stamp and embossed substrate indicate that the patterns were transferred consistently across the whole surface. The cross sectional examination of the surfaces in ESEM on both Si master (Fig. 34), and embossed PMMA confirms the features fabricated on these surfaces (Fig. 35).

Fig. 34: ESEM image of V-grooved Si master [39]
Roughness measurements on SU8 based shallow grooved stamp and embossed PMMA indicate smoother surfaces can be made by utilizing SU8 resist (Fig. 36). As the stamp was not etched into a hard surface like Si and the pattern was only defined on SU8 resist which after a hard bake becomes more defined and hardened, the roughness of all the sides were similar and was smoother (maximum 1.3 nm) than deeper V-groove stamps fabricated using the silicon micromachining process.
After five of each coupon was added into the reactor they were allowed to sit in the algae culture for approximately 2 days before the pumps were started. On Day 0 (which is the day the pump was started) one of each PMMA kind was taken out for ESEM analysis. The images reveal there were algal film deposits prominent on deep V-grooved surfaces as in Fig. 37. The algal flocks are coming together and forming a close network between them while embedding within the grooves and going over the troughs on the deep V-grooved surfaces as seen in Fig. 37. In Fig. 38, EPS like structure can be noticed on the deep V-groove surface and seems like EPS prefers to hang onto the slope of the deep V-grooves. Although this was not seen on other surfaces and needs to be further investigated, this could be telling us that the EPS may prefer deep V-grooved surfaces. Since EPS gives mechanical stability to form biofilms and mediate their adhesion to surface to form cohesive networks, if this was proved to be the case from further investigations these types of features can be adapted and tested in other studies related to cells and tissues [35]. Similar V-grooved surface with 5 µm depth was reported in literature and the group has investigated osteoblast cells which resulted in focal adhesion and therefore significant alignment within grooves [60]. Although the cell type is different in this case, this kind of work should be done with deeper structures to see if depth of feature can influence adhesion of such cells.
Fig. 37. ESEM image of algal biofilm growth on Deep V-groove PMMA surface on Day 0 [39]

Fig. 38. ESEM images of algal biofilm growth on Deep V-groove PMMA where EPS attachment is visible on Day 0 [39]
Fig. 39: ESEM image of algal biofilm growth on smooth PMMA surface on Day 0 [39]

Fig. 40: ESEM images of algal biofilm growth on vertical lines shallow groove PMMA surface on Day 0 [39]
For comparison, on the smooth PMMA surface (Fig. 39) there are few individual flocks of algae are seen sitting on distinct spots but they are not forming a close network as on the deep V-grooved surfaces. Also algal flocks seem to be distinctly sitting on vertical line shallow groove substrate and look similar to that of flat surface (Fig. 40) and no network of algae is visible. This could be because the depth and the width of the lines are too small and are not on the order of the more prominent algal species’ sizes (S. obliquus size: 10-15 µm) hence presumably the lines were not noticed by the algae. Therefore from these analyses it can be concluded that the depth size seems to matter with algal species size and they seem to prefer embedding into features that are about the same size or bigger than the prominent algal species size.

Fig. 41: Deep V-groove on Day 4 after scrapping
On day 4, all the coupons were completely covered and the pattern underneath were not visible when inspected under ESEM. However after scrapping off the film for the dry mass analysis, the coupons were inspected under ESEM again. The Fig. 41 shows deeper V-groove on Day 4 after scrapping and this show considerable amount algal species are left behind within the deep V-grooves as compared to a shallow vertical line substrate surface (Fig. 42). It is also clear that embedding of algae initiated the attachment of more algal species during the initial days. The vertical line shallow grooves and horizontal line shallow grooves both looked similar where embedding was very less because the size of grooves were negligible for algae. Therefore scrapping off for algal mass analysis had taken out almost all the algal film out from the shallower line patterns. It is expected that since the deeper grooves leave the algal species embedded within the deep grooves, the regrowth on such surfaces will be much faster compared to shallower patterns or flat PMMA surfaces. If this prediction is proved with further studies, this can allow us to regrow algal biomass in a repeated fashion.
On Day 10 the total algal biomass grown on these surfaces were weighed after scraping the biomass out of all the surfaces and plotted for algal productivity in g/m² day (Fig. 43). Compared to the smooth PMMA control surfaces, algal biofilms grown on deep V-groove embossed PMMA surfaces had a statistically significant increase in biomass production at the 95% confidence level. This trend was repeatedly observed over three runs. The higher algal biofilm productivity on the deeper V-grooved surface could be attributed to the presence of larger surface area for attachment of the algal film. There is a 48% more surface area for attachment on the deeper V-groove surface over a surface with no pattern and this has produced 101% more algae. Similarly compared to the vertical shallow grooved surface, deep V-grooved surface had a 45% more surface area for attachment but produced 109% more algae as listed on Table 5.

Table 5: Surface area increase and productivity increase between the surfaces

<table>
<thead>
<tr>
<th></th>
<th>Increase in Surface Area</th>
<th>Increase in Productivity</th>
</tr>
</thead>
<tbody>
<tr>
<td>Deep V to Smooth</td>
<td>48%</td>
<td>101%</td>
</tr>
<tr>
<td>Vertical Shallow to Smooth</td>
<td>2.50%</td>
<td>-3.90%</td>
</tr>
<tr>
<td>Deep V to Vertical Shallow</td>
<td>45%</td>
<td>109%</td>
</tr>
</tbody>
</table>
Vertical shallow grooves had a 2.5% more surface area than the smooth surface, and their productivity of biofilms was not significantly different from the smooth surface when statistically compared.

![Deep V-groove surface cross section and vertical shallow groove cross section](image)

Fig. 44: a) Deep V-groove surface cross section and b) vertical shallow groove cross section

The reason for the doubling effect of productivity on deep V grooved surfaces can be attributed to the availability of more surface area as in Fig. 44a), compared to shallow grooves as in Fig 44b). Furthermore the depth will allow more insertion of algae within the deeper features. In addition to these two reasons, there could be other reasons that are contributing to the enhanced growth on deep grooves. In order to test what other reasons may influence higher productivity, a comprehensive study on biofilm thickness and everyday productivity tests will be needed. Moreover only two feature depths were studied in this research with ~20 µm performing better than the ~1.5 µm depth microstructures. However the optimum feature depth that can enhance biofilm growth will still have to be further investigated. In addition to that if we take into account the large amount of embedded algae left behind within the deep V-grooves after scrapping as in Fig 41, the deeper V-groove substrates could have made more dry mass than what is reported in productivity graph (Fig 43). The flat PMMA surface, horizontal and vertical line shallow patterns are statistically similar in their algal productivity values. This could be because the depths of horizontal and vertical shallow groove patterns were very small (1.5 µm) compared to the algal species size. Therefore they tend to treat them just like the smooth surface.
Shallow grooved surfaces were tested with lines going vertical and horizontal with respect to the air diffuser. These two different lines did not provide much difference in the productivity tests (Fig. 43) or when inspected under the electron microscope. Therefore it is possible that when feature depths were small in size (~1.5 µm), the direction of grooves were not an issue. However the deep V grooves surfaces were only tested with grooves going vertical inside the reactor. As in Fig 45 schematic illustration, compared to vertical deep V-grooves, horizontal deep V-grooves should also be tested in further studies to come to a definite understanding of if the direction of grooves inside the photo bioreactor matters to the algae production.
Chapter 5
Cell Migration

5 Micro-Structured Surfaces for Cell Migration

Guidance and response of individual cells to topographical cues has been extensively studied but very little is known about the response of groups of cells to topographic cues. Collective cell migration is the fundamental process for many cell and tissue progression such as embryo development and adult organ regeneration [19]. Understanding the groups of cells’ response to micro-structured surfaces therefore helps develop knowledge on how these cells behave in general to topographical cues vs. flat surface. For this purpose grooved surfaces were made with photolithography on a Si stamp. Patterns from Si stamp were replicated and studied for cell behavior by collaborators. Most of the collaboration work on cells and their behavior that are reported in this chapter has been peer-reviewed and accepted for publication in December 2013 in Proceedings of the National Academy of Sciences of the United States of America journal (Early edition available: Londono, C. et al. doi: 10.1073/pnas.1321852111) and has been included here with permission from co-authors [19].

5.1 Design and Fabrication

As a first step to fabricate a stamp, a photomask (5” x 5”) was designed using L-edit software. Features were 2 μm wide lines with 2 μm spacing (Fig. 46). Patterns on one side of the mask were designed with lines going vertical and the other half of the mask was designed with lines going horizontal. The mask had alternating flat and patterned areas. It also had markers or alignment features to align the wells of a microplate with patterned surface. In some studies wells were to be arranged fully covering the patterned surface underneath and in some other studies only half of the patterns were to be underneath the well while other half sits on a smooth surface. This way of arranging the patterns to carefully align with the microwells would allow us to study cells’ interaction to interfaces (between smooth and patterned surface). Markers were also added for aligning a whole well to be on half of the patterned surface with other half being on a flat surface. Since the resolution of features is very small and in order to resolve such small features a chrome-glass mask was decided to be printed. The design was submitted through
online and it was printed and shipped back from Micro & Nanofabrication Facility, University of Alberta.

Fig. 46. Schematic representation of Glass-Cr mask designed with 2 µm lines and alignment features of full well and half well arrangements to make custom microplate

The master was made out of Si wafer. A new 4” Si wafer was first cleaned with acetone sonication for 3 min, IPA (isopropyl alcohol) rinse for 1 min and de-ionized water rinse for 1 min and then the wafer was blow dried with nitrogen. Dehydration bake was then followed for 2 minutes at 105 °C. After cooling down the wafer, adhesive primer hexamethyldisilazane (P20-HMDS) was spun coated on the wafer at 4000 rpm for 40s followed by a positive resist coating (MICROPOSIT® S1811® PHOTO RESIST) at the same speed and time [65]. The resist-coated wafer substrate was soft-baked to remove solvents at 105 °C on a hotplate for 2 min.

The photomask was placed on the mask aligner (Suss MicroTec MA6 Mask Aligner) followed by loading the resist-coated wafer into the machine. The machine was then enabled to expose the wafer under UV (365 nm wavelength, 16.9 mW/cm² intensity) in hard contact mode for 10 s. Wafer was then unloaded from the mask aligner and developed in MF-321 (MICROPOSIT® MF® -321DEVELOPER) with gentle agitation for 40-50 sec or until the resist clears out in the
exposed areas followed by DI water rinse for about the same time. Wafer was inspected under an optical microscope to ensure if resist had been removed from all the exposed areas of the substrate. The substrate was hard-baked at 105 ºC on a hotplate for 2 min in order to harden the resist to improve adhesion and thereby etching of the wafer.

Dry etching was carried out using inductively coupled plasma reactive ion etcher (Trion Phantom II RIE/ICP System). The recipe for etching the Si stamp was SF₆, O₂, CHF₃ and He etchants, with a flow rate of 30 sccm, 20 sccm, 12 sccm and 10 sccm respectively and had a pressure of 100 mTorr and RIE RF power of 120 W (Recipe 1: Xiao Sun and James Dou Thesis work). The etch rate was observed to be slow at the beginning and then increased, which was approximated to be 400nm/min with the above recipe. The CHF₃ etchant was particularly useful for silicon oxide etching and here it was beneficial for etching any native oxide on the Si. A modification to recipe 1 was carried out with smaller CHF₃ and He etchants flow that had 5 sccm and 5 sccm flow rates respectively (Recipe 2).

Profilometer measurements were done in the wider spacing (alignment features) in between each etching runs until the desired or close to desired depths were achieved. Three substrates were etched for different depths, at 30 s, 75 s and 300 s resulting in ~180 nm, ~600 nm and ~3.13 μm depths in the wider spacing respectively. After completing the etching process, the remaining resist layer was removed by immersing the master plate in acetone. Before pouring the PDMS, the master plate was rinsed in DI water. The Fig. 47 depicts the process of making these Si based microplate stamps. At the end of the process atomic force microscopy measurements were primarily done to characterize the final surface.

5.2 Results and Discussion

The atomic force measurements (Veeco Dimension 3100 AFM) were carried out on the Si stamps that were fabricated for this purpose to understand the step height of the features. Below
is an AFM result of a 630 nm deep Si master fabricated for this study (Fig. 48). The fabricated grooves were ~2 µm wide and 4 µm in pitch.

![Figure 48](image1.png)

**Fig. 48.** Step-height (left) and AFM image (right) of Si stamp with etch recipe 1

This was fabricated with the first recipe. However, a more anisotropic etch-profile was achievable with the second recipe that had a smaller flow rate for CHF$_3$ and He as in Fig 49. This etching recipe had similar etch rates as the first recipe.

![Figure 49](image2.png)

**Fig. 49:** Step-height (left) and AFM image (right) of grooved Si stamp etched with recipe 2

Table 6: Dry etching time and etch depths achieved with etch recipe 1

<table>
<thead>
<tr>
<th>Etch Time (s)</th>
<th>Etch depths (µm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>30</td>
<td>180</td>
</tr>
<tr>
<td>75</td>
<td>630</td>
</tr>
<tr>
<td>300</td>
<td>3130</td>
</tr>
</tbody>
</table>
The step height measurements taken on different spots along the stamp were averaged to get the mean depth on each Si stamp. The three Si stamp fabricated with different dry etching times for different depths are given on Table 6. The collaborators carried out PDMS (polydimethyl siloxane) replication process. Scanning electron microscope imaging was done on the PDMS replica of the shallower Si master as in Fig 50. The depths of these replicas seem to be shallower than the actual depth of the master stamp fabricated on Si.

![Fig. 50. SEM image of PDMS replica made from Si stamp](image)

The vertical lines going parallel to the flat interface is given in Fig. 51 below. In this particular design the grooves were 531 nm.

![Fig. 51. Step height and AFM image of the interface of parallel grooves and flat surface](image)
Migration paths of individual ARPE-19 (Human retinal pigment epithelial cells) and BJ (Human foreskin fibroblast) cells at both sparse and confluent densities on flat and grooved topography were tested on the PDMS replicas of the fabricated Si stamps. The ARPE-19 cells form confluent epithelial monolayer sheets and BJ cells are non-epithelial cell type in which continuous cell-cell junctions are not formed within the sheet [28].

Fig. 52. Tracking of each cell nuclei which is guided on the grooved surface and randomly oriented on flat surface. The color wheel shows the different angle with respect to grooves (predominant blue lines and yellow lines on the bottom half of the image shows that those cells were either ~0º or ~180º (ie. parallel) with respect to the grooves

High Throughput microscope Microxpress (from Molecular Devices) were used in tracking individual cell’s path on grooved and flat surfaces as in Fig. 52 [19].
Fig. 53: Tracking of cells show cells crossed the flat-groove interface when the grooves were perpendicular to the interface as in (A). Cells on a surface that are parallel to the interface did not cross the interface but guided by those cells that were within the grooves as in (B). Guided cells are marked in red and non-guided grooves are in blue. Image adapted from [19].

On a more comprehensive study of half well alignment (well sitting on flat and grooved surface) the results show that those cells on flat region are being guided by those cells that are on the grooved surface. The results suggest both cell types (ARPE and BJ) migrated parallel to the grooves with a highest distribution of guidance angles between -25 to 25° (Fig. 53) of a microplate with grooves parallel to the direction of grooves [19]. At sparse densities, 52.1 % of ARPE-19 and 70.7 % of BJ cells migrated in a direction parallel to the grooves and in confluent densities 59.1 % of ARPE-19 and 80.6 % of BJ cells were within the -25 to 25° distribution peak [19]. On grooves that are perpendicular to the flat and grooved interface, cells start to crossover the interface and were clearly visible to be guided by the grooves in the direction of the grooves (Fig. 53A). After they started guiding into the grooves, even on flat region towards the interface the cells started to be guided for several rows of cells. This is because as the cells started migrating into the grooves, other cells that are on the vicinity of the guided cells are being pushed or pulled in the same direction.

On the grooves that are parallel to the flat interface, there is no visible crossing of cells into the grooves but guidance is still observed which could mean that the cells do not have to be in direct contact with the grooves to be guided on the flat surface but the propagation of guided signal can
make cells in the close proximity of the interface to be guided (Fig. 53B). This is called non-autonomous guidance which means signals transmitted between neighbors to act on cells with which they are not in direct contact. In these images red tracking line implies the cells or nuclei of cells, which were guided in the direction of the grooves whereas blue track lines are of those cells that were not guided by the grooves [19]. This data suggests that grooves are capable of guiding cells that are both sparse and confluent cell densities in the epithelial and fibroblast cell types tested. In addition to that, grooved surfaces increased the confluent cell velocity and persistence, and increased intercellular coordination. In addition to confirming model results, the custom designed Si stamps also helped in concluding when cells are in constrained space, cell to cell cooperation during cell group migration is not due to tension based forces at the cell to cell junction as often described but by an emerging property called mechanical exclusion interaction within the collective cells. Further exploration of this type of interaction will have to be carried out in order to derive a complete understanding of cell cooperation during group cell migration in a constrained and textured space.
Chapter 6
Conclusion

6 Conclusion

6.1 Concluding Remarks

Microalgae are currently of interest as a potential source of energy in the form of biofuel due to their high oil content and ability to rapidly produce biomass. Growing algae as a biofilm inside photobioreactors are preferred over growing them in suspension because it can potentially be produced in an energy efficient manner. While growing them as films, surface on which they can be grown becomes a topic of interest. Tissue engineering and biofouling fields have studied micro texturing of surfaces extensively and proved that cells of any organism respond profoundly to surface cues. Similarly by texturing the surface, we wanted to see if structuring a surface utilizing photolithography and hot embossing methods can enhance the growth of algae to form biofilms.

From this work, we conclude that using photolithography, etching processes along with hot embossing technique, consistent grooves in material that promote algal biofilm growth can be fabricated. Silicon based stamps are primarily used for making these patterns on a hard stamp because the features are in the micrometer scale and are capable of being made with well utilized photolithography and etching processes. The Jenoptik HEX 02 hot embosser was used in transferring microstructures from Si stamps into PMMA substrates. A parametric analysis was carried out to understand some of the factors that can affect the quality of embossed features. Force, temperature and emboss hold time were chosen to be tested one at a time in parametric analysis while keeping all other settings identical. Results suggest that in order to get the same feature depth of Si stamp, the emboss hold time had to be increased with a decreasing force. Temperature did not affect the quality of the PMMA in terms of its feature depth, between the tested temperature range (115 °C – 145 °C). However this test confirmed that the force was affecting the flow of PMMA. Large force pushes the PMMA to flow into the features whereas smaller force did not give the desired results at the chosen temperatures. Longer emboss hold time was required (10 min or more) while applying a smaller force (5kN) and a temperature within 115 to 145 °C in order to efficiently transfer 20 µm deep structures from a silane treated
Si based stamp. However the depth of the stamp was easily achievable within a short period of emboss hold time (4 min) since the flow of PMMA was increased with a higher punching force of 10kN. Although the silane treatment would allow moderately faster replication at higher forces, since the Si stamps are brittle, the stamp will degrade at a faster rate compared to using smaller emboss forces with the same stamp. Since the viscosity and therefore the flow of PMMA was the deciding factor for choosing an emboss force and hold time as long as the temperature is above Tg, the PMMA e-beam resist which is in low viscous form at room temperature was combined with PMMA casting sheet to allow faster flow of PMMA into the structures studied. This had resulted in a good quality replication at a smaller force of 5kN within a shorter embosses hold time (100 s). By combining hot embossing and imprinting techniques micro-structured surfaces can be fabricated without damaging the stamp and this process can be done in less time.

In the biofilm study it has been shown from characterization results and productivity tests that smooth and shallow grooved PMMA surfaces do not promote algal biofilm growth however patterning the PMMA surface with deeper V-grooved structures had statistically higher biomass productivity. Surface structuring with deep V-groove patterns that are on the same size scale in depth (~20 µm) as the prominent algal species in the media (S. obliquus size: 10-15 µm) has resulted in higher biomass productivity. On the deep V-grooved PMMA surfaces network formation of algal species were observed whereas shallow grooves and completely smooth surfaces did not show this trend. The smooth surface, horizontal and vertical shallow (1.5 µm) groove patterns were statistically similar in their algal productivity. On the shallow line groves, the features are too small and are not on the order the more prominent algal species therefore it is probable that the algae did not notice them. Compared to a surface with no pattern, there was a 48% more surface area for attachment on the deep V-grooved surface and the higher productivity could be attributed to this availability of more surface area for initial attachment. In addition to that deep structures enabled algae to embed within the features. However since there was a 101% more production on deep V-grooves compared to smooth PMMA surfaces, there should also be other factors contributing to the doubled productivity results.

After scrapping the biofilm out of the surface for dry biomass analysis on Day 4, algal species were still left embedded within the deep V-grooves. This would also mean that more than what is
reported on productivity tests could be achieved with deep V-grooved surface. When algae are left behind inside the grooves after scrapping they can enable more growth when utilized for regrowing algal biofilms on repeated biomass production. When the direction of shallow grooves with respect to the air diffuser (flow of air) tested, there was no difference between the vertical shallow grooves and horizontal shallow grooves. They both behaved identical to each other, showing that the directions of shallow grooves that are on the order of ~1.5 µm depth do not influence the algal productivity but deeper groove surfaces may or may not influence the productivity if the grooves’ orientation with respect to air diffuser is tested in further studies.

For cell guidance study, custom-made microplates were designed and fabricated on Si stamp with grooves that are perpendicular and parallel to flat interfaces. Photolithography and dry etching processes were utilized in making a stamp in silicon with different depths. These micro patterns were characterized in AFM. Using the custom made micro-plates; the patterns were fabricated on suitable PDMS polymer and ARPE-19 epithelial cells that form cell-cell junction and BJ cells at both sparse and confluent densities were tested for cell guidance. The microplates were useful in concluding that the contact guidance was visible on flat surfaces that had an interface that is perpendicular to the grooves which allowed cells to cross over the interface and to extend their guidance even on the flat surface. On the other hand, cells on grooves that were parallel to the interface did not cross the interface but were still guided by those that were on grooved surface in the same direction as the grooves confirming that guidance not necessarily have to be contact guidance and it could be a non-autonomous guidance. Therefore flat to grooved interface has showed signal propagation occurring between neighboring human cells triggered by mechanical exclusion interaction within collective systems.

6.2 Future Work

Micro-structured surfaces for algal biofilm and cell guidance studies were fabricated in this work and the results have contributed in understanding and confirming existing knowledge, and developing new knowledge on how algae and human cells behave on the fabricated structured surfaces and interfaces. Moreover opportunities for future research arise from this work as well.

The PMMA resist and PMMA casting sheet combination in embossing should be further investigated as this method was only developed in the later part of this work. It is important to check if the adhesiveness of PMMA resist to PMMA casting sheet is permanent and does not
degrade over time after embossing or if methyl methacrylate monomer treatment would be necessary. This type of monomer treatment is usually done when bonding PMMA thin film with PMMA casting sheet to increase thickness of the sheet [61]. Additionally, the temperature range that was studied in the parametric test was 115 – 145 °C and within this range the flow of PMMA or the viscosity did not seem to be affected. The viscosity of PMMA can be decreased if the temperature is further increased. Therefore embossing at a larger temperature as close to 160°C can also be carried out to see if the temperature dependence and viscosity affect the resulting replica.

Among the tested surfaces, deep V-grooved surface has produced double the amount of algae compared to shallow grooved and smooth PMMA surfaces. Availability of more surface area and the depth can be two contributing factors to the high biomass production. However algal species were embedded inside the deep V-grooves after scrapping. This would mean that more algae than what was reported on productivity tests could be produced with deep V grooved surfaces. Therefore in order to test what other reasons may influence this higher productivity, a comprehensive study on biofilm thickness and everyday productivity tests will be needed. In the meantime since algae were left behind after scrapping the surface, deep V grooved surface can be employed to re-growing biofilms for repeated runs. A regrowth study can be done further on deep grooves vs. smooth surfaces to confirm this prediction. Furthermore deep V-grooved surfaces were only made with vertical line mask and when they were placed inside the reactor the grooves were perpendicular to the air diffuser. However in order to test if the deep grooves’ orientation inside the reactor impact the growth of algae, the grooves with similar dimensions will have to be made using horizontal line mask which will then allow the grooves to be parallel to the air diffuser inside the reactor. More fabrication and productivity analysis will be needed in this work to learn if orientation of deep V grooves impacts the productivity results.

Furthermore in this research, focus was primarily given to one type of polymer material (PMMA). However other thermoplastic polymers such as polycarbonate can also be embossed and tested for algal biofilm growth and productivity by following similar methods. Other surfaces that performed well in producing algal biomass compared to smooth PMMAs, should also be embossed to see if similar micro-patterning further enhances or inhibit algae attachment. These results will eventually help to come to a complete understanding of algae’s behavior on grooved surfaces.
If micro-structuring the thermoplastics with specific features enhance algal attachment and produce thick algal biofilms, a more comprehensive and a large scale production system will have to be investigated to take this to the next level. Hot embossing in general is a scalable technology but some machines are only intended for research purposes. However if micro structuring on thermoplastics or other materials can enhance algal attachment and growth of biofilm, they can be made with roll-by-roll embossing or 3D printing.

In the cell guidance study, various structures with different dimensions in terms of pitch spacing can be fabricated to study the complete mechanism by which the signal propagation happens. This will have to be further investigated in order to provide complete understanding into tissue formation for regenerative medicine applications.
References


[53] Standard process PMMA, Jenoptik Germany, (June 2006).


Appendix A
Publications

Conference Proceeding and Presentation

Peer-reviewed Accepted Paper for Publication

Conference Presentation
Appendix B
Hot Embossing Recipes

Preliminary Embossing Recipes

145 C, 5kN, 120s

Open File Protocol(New,View,Print=0(0,1,2))
Open File Measure()
Initialize ForceControl(true/false=0)
Heating(Top=176.0°C,Bottom=169.0°C)
Close Chamber()
Evacuate Chamber()
Position relative(Position=13.00000mm,Velocity=50.00000mm/min,MaxForce=2000N)
Show Chart Window(Show/Hide=01/0)
Show Chart Window(Show/Hide=11/0)
Touch Force(Force=50N)
Temperature >={Temperature=128.0deg,Channel=12)
Heating(Top=145.0°C,Bottom=145.0°C)
Force - Force controled(Force=5000N,Velocity=0.23000mm/min)
Wait Time(Time=120.00s)
Cooling(Top=65.0deg,Bottom=65.0deg)
Temperature <=={Temperature=85.0deg,Channel=12)
Temper(Top=80.0deg,Bottom=80.0deg)
Wait Time(Time=60.00s)
DemoldingAdv(Stretch=0.60000mm,Velocity=0.40000mm/min)
Open Chamber fast()
Close File Measure()
Show Chart Window(Show/Hide=01/0)

120 C, 5kN, 240s

Open File Protocol(New,View,Print=0(0,1,2))
Open File Measure()
Initialize ForceControl(true/false=0)
Heating(Top=159.0°C,Bottom=145.0°C)
Close Chamber()
Evacuate Chamber()
Position relative(Position=13.00000mm,Velocity=50.00000mm/min,MaxForce=2000N)
Show Chart Window(Show/Hide=01/0)
Show Chart Window(Show/Hide=11/0)
Touch Force(Force=50N)
Temperature >={Temperature=100.0deg,Channel=12)
Heating(Top=120.0°C,Bottom=120.0°C)
Force - Force controled(Force=5000N,Velocity=0.23000mm/min)
Wait Time(Time=240.00s)
Cooling(Top=65.0deg,Bottom=65.0deg)
Temperature <=={Temperature=85.0deg,Channel=12)
Temper(Top=80.0deg,Bottom=80.0deg)
Wait Time(Time=120.00s)
DemoldingAdv(Stretch=0.60000mm,Velocity=0.40000mm/min)
Open Chamber fast()
Close File Measure()
Show Chart Window(Show/Hide=01/0)
120 C, 10kN, 120s

Open File Protocol(New,View,Print=0[0,1,2])
Open File Measure()
Initialize ForceControl(true/false=0)
Heating(Top=159.0°C,Bottom=145.0°C)
Close Chamber()
Evacuate Chamber()
Position relative(Position=13.000000mm,Velocity=50.000000mm/min,MaxForce=2000N)
Show Chart Window(Show/Hide=01/0)
Show Chart Window(Show/Hide=11/0)
Touch Force(Force=50N)
Temperature >=[Temperature=100.0deg,Channel=12]
Heating(Top=120.0°C,Bottom=120.0°C)
Force - Force controled[Force=10000N,Velocity=0.230000mm/min]
Wait Time(Time=120.00s)
Cooling(Top=65.0deg,Bottom=65.0deg)
Temperature <=[Temperature=85.0deg,Channel=12]
Temper(Top=80.0deg,Bottom=80.0deg)
Wait Time(Time=60.00s)
Demolding()
Open Chamber fast()
Close File Measure()
Show Chart Window(Show/Hide=01/0)

Initial embossing for algal film study recipe: 10kN, 140 C, 600s

Open File Protocol(New,View,Print=0[0,1,2])
Open File Measure()
Close door()
Initialize ForceControl(true/false=0)
Heating(Top=154.0°C,Bottom=147.0°C)
Close Chamber()
Evacuate Chamber()
Position relative(Position=12.000000mm,Velocity=50.000000mm/min,MaxForce=2000N)
Show Chart Window(Show/Hide=01/0)
Show Chart Window(Show/Hide=11/0)
Touch Force(Force=300N)
Temperature >=[Temperature=140.0deg,Channel=12]
Heating(Top=140.0°C,Bottom=140.0°C)
Force - Force controled[Force=10000N,Velocity=1.000000mm/min]
Wait Time(Time=600.00s)
Cooling(Top=20.0deg,Bottom=20.0deg)
Temperature <=[Temperature=65.0deg,Channel=11]
Temper(Top=60.0deg,Bottom=60.0deg)
Wait Time(Time=600.00s)
Demolding()
Open Chamber fast()
Open door()
Close File Measure()
Show Chart Window(Show/Hide=01/0)
Parametric study recipes

Wait Time Test recipe: 130 C, 5 kN, 310s

```
Open File Protocol New View Print=0[0,1,2]
Open File Measure()
Close door()
Initialize ForceControl(true/false=0)
Heating(Top=145.0°C,Bottom=140.0°C)
Close Chamber()
Evacuate Chamber()
Position relative(Position=12.00000mm,Velocity=50.00000mm/min,MaxForce=2000N)
Show Chart Window(Show/Hide=01/0)
Show Chart Window(Show/Hide=11/0)
Touch Force(Force=300N)
Temperature >= [Temperature=130.0 deg, Channel=12]
Heating(Top=130.0°C,Bottom=130.0°C)
Force - Force controled(Force=5000N,Velocity=1.00000mm/min)
Wait Time(Time=310.000s)
Cooling(Top=20.0 deg,Bottom=20.0 deg)
Temperature <= [Temperature=65.0 deg, Channel=11]
Temperature(Top=60.0 deg,Bottom=60.0 deg)
Wait Time(Time=100.000s)
Demolding() Open Chamber fast()
Open door()
Close File Measure()
Show Chart Window(Show/Hide=01/0)
```

Temperature study recipe: 100s, 10kN, 145 C

```
Open File Protocol New View Print=0[0,1,2]
Open File Measure()
Close door()
Initialize ForceControl(true/false=0)
Heating(Top=145.0°C,Bottom=140.0°C)
Close Chamber()
Evacuate Chamber()
Position relative(Position=12.00000mm,Velocity=50.00000mm/min,MaxForce=2000N)
Show Chart Window(Show/Hide=01/0)
Show Chart Window(Show/Hide=11/0)
Touch Force(Force=300N)
Temperature >= [Temperature=130.0 deg, Channel=12]
Heating(Top=145.0°C,Bottom=145.0°C)
Force - Force controled(Force=10000N,Velocity=1.00000mm/min)
Wait Time(Time=100.000s)
Cooling(Top=20.0 deg,Bottom=20.0 deg)
Temperature <= [Temperature=65.0 deg, Channel=11]
Temperature(Top=60.0 deg,Bottom=60.0 deg)
Wait Time(Time=100.000s)
Demolding() Open Chamber fast()
Open door()
Close File Measure()
Show Chart Window(Show/Hide=01/0)
```
Emboss with Resist PMMA and PMMA sheet combined recipe: 5kN, 130 C, 100s

```
[Open File Protocol]New View Print=0,1,2]
Open File Measure()
Close door()
Initialize ForceControl(true/false=0)
Heating(Top=135.0°C, Bottom=130.0°C)
Close Chamber()
Evacuate Chamber()
Position relative(Position=12.000000mm/Velocit=50.000000mm/min/MaxForce=2000N)
Show Chart Window(Show/Hide=01/0)
Show Chart Window(Show/Hide=11/0)
Touch Force(Force=300N)
Temperature :=(Temperature=130.0deg, Channel=12)
Heating(Top=130.0°C, Bottom=130.0°C)
Force - Force controled(Force=5000N, Velocity=1.000000mm/min)
Wait Time(Time=100.000s)
Cooling(Top=20.00deg, Bottom=20.00deg)
Temperature :=(Temperature=65.0deg, Channel=11)
Temperature(Top=60.00deg, Bottom=60.00deg)
Wait Time(Time=100.000s)
Demolding()
Open Chamber fast()
Open door()
Close File Measure()
Show Chart Window(Show/Hide=01/0)
```